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<b>(54) Title:</b> HUMANIZED CHIMERIC ANTI-ICAM-1 ANTIBODIES, METHODS OF PREPARATION AND USE  <b>(57) Abstract</b>  <p>The present invention discloses humanized chimeric antibodies which are capable of binding to the intercellular adhesion molecule ICAM-1. Specifically, disclosed are humanized anti-ICAM-1 antibodies of the IgG1, IgG2, and IgG4 subtype. These antibodies are useful in treating specific and non-specific inflammation, rhinoviral infection, HIV infection, the dissemination of HIV infected cells, and asthma. In addition, the humanized antibodies disclosed can be useful in methods of diagnosing and localizing sites of inflammation and infection and tumors expressing ICAM-1.</p>		

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**HUMANIZED CHIMERIC ANTI-ICAM-1 ANTIBODIES, METHODS  
OF PREPARATION AND USE**

**Field of the Invention:**

5       The present invention relates to a chimeric antibody molecule, and especially a humanized chimeric antibody molecule, having specificity for an antigenic determinant of Intercellular Adhesion Molecule 1 (ICAM-1), to a process for its production using recombinant DNA technology and to its therapeutic use.

10       In the present application, the term "chimeric antibody molecule" is used to describe an antibody molecule having heavy and/or light chains comprising at least the variable regions of heavy and/or light chains derived for one immunoglobulin molecule linked to at least part of a second protein. The second protein may comprise additional antibody constant regions domains derived from a different immunoglobulin molecule or a non-immunoglobulin protein. The term "humanized chimeric antibody molecule" is used to describe a molecule having heavy and light chain variable region domains derived from an immunoglobulin from a non-human species, the remaining immunoglobulin constant region domains of the molecule being derived from a human immunoglobulin. The abbreviation "MAb" is used to indicate a monoclonal antibody.

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      The present invention also relates to the use of chimeric antibodies capable of binding to ICAM-1 to inhibit intercellular adhesion of cells of granulocyte or macrophage lineage. The use of such molecules provides a method for the treatment of specific and non-specific inflammation.

The present invention also relates to chimeric antibody capable of binding ICAM-1 in the treatment of viral, and particularly rhinoviral disease.

5 The invention also relates to therapeutic and prophylactic methods for suppressing the infection of leukocytes with HIV, and particularly with HIV-1, in an individual who is exposed to HIV or effected by HIV, and is thus in need of such suppression through the administration of a chimeric antibody capable of binding ICAM-1 . It therefore provides a therapy for diseases, such as AIDS (Acquired Immunodeficiency Syndrome) which are caused by the HIV virus.

10 The invention also relates to a therapeutic method for suppressing the migration of HIV-1 infected cells from the circulatory system using chimeric antibodies capable of binding ICAM-1 . It therefore provides a therapy for diseases, such as AIDS (Acquired Immunodeficiency Syndrome) which are caused by the HIV-1 virus.

15 The present invention relates to the use chimeric antibodies capable of binding ICAM-1 in the treatment of asthma.

#### Background of the Invention

##### A. Humanized antibodies

20 Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, (Fab')<sub>2</sub> and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the free end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with  
25 immunoglobulins.

Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, have been hindered by the polyclonal nature of natural immunoglobulins. A significant step towards the realization of the potential of immunoglobulins as  
30 therapeutic agents was the discovery of techniques for the preparation of monoclonal antibodies of defined specificity (Kohler *et al.*, *Nature* 265:295-

497 (1975)). However, most MABs are produced by fusions of rodent spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins. There are very few reports of the production of human MABs.

5 Since most available MABs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MABs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness. In  
10 practice MABs of rodent origin may not be used in a patient for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions.

Proposals have therefore been made for making non-human MABs less antigenic in humans. Such techniques can be generically termed  
15 "humanization" techniques. These techniques generally involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

In particular one procedure which has been proposed for the preparation of humanized antibodies is the so-called chimerization procedures.

20 Such chimerization procedures involve production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody is linked to constant domains derived from another antibody. Some early methods for carrying out such a chimerization procedure are described in EP-A-0120694 (Celltech Limited), EP-A-0125023  
25 (Genentech Inc. and City of Hope), EP-A-01714906 (Res. Dev. Corp. Japan), EP-A-0173494 (Stanford University), EP-A-0194276 (Celltech Limited). The latter Celltech application also shows the production of an antibody molecule comprising the variable domains of a mouse MAB, the CH1 and CL domains of a human immunoglobulin, and a non-immunoglobulin derived protein in  
30 place of the Fc portion of the human immunoglobulin.

## B. Leukocyte Attachment and Functions

Leukocytes and granulocytes must be able to adhere to cellular substrates in order for an inflammatory response to occur and to properly defend the host against foreign invaders such as viruses, bacteria, and allergens. This fact has become evident from two converging lines of research.

The first line of research involves studies of leukocyte membrane proteins (Wallis, W.J., *et al.*, *J. Immunol.* 135:2323-2330 (1985); Mentzer, S.J., *et al.*, *J. Cell. Physiol.* 126:285-290 (1986); Haskard, D.O., *et al.*, *J. Immunol.* 137:2901-2906 (1986); Harlan, J.M., *et al.*, *Blood* 66:167-178 (1985)). Of particular importance to the process of cellular adhesion is a family of leukocyte membrane proteins known as the "CD18" family or complex. This family consists of three heterodimers (known as "Mac-1," "LFA-1," and "P150,90"), all of which share a common subunit (known as the  $\beta$  subunit) and a unique subunit (known as the  $\alpha$  subunit) (Springer, T.A., *et al.*, *Immunol. Rev.* 68:111-135 (1982); Springer, T., *et al.*, *Fed. Proc.* 44:2660-2663 (1985); Keizer, G., *et al.*, *Eur. J. Immunol.* 15:1142-1147 (1985); Sanchez-Madrid, F., *et al.*, *J. Exper. Med.* 158:1785-1803 (1983)).

Monoclonal antibodies against the CD18 family of leukocyte membrane proteins, by acting as antagonists of these proteins, inhibit a multitude of leukocyte adhesion dependent events *in vitro*. This includes the ability of granulocytes to aggregate in response to appropriate stimuli, the ability of granulocytes to attach to protein coated plastic, the ability of granulocytes to migrate in 2-dimensional agarose assays, and the ability of granulocytes to attach to endothelial cells.

The second line of research results from studies involving individuals, who, due to an inherited flaw in the gene encoding for the common subunit of the CD18 family of leukocyte adhesion molecules, are unable to express any of these adhesion molecules on the surfaces of their cells. Such individuals are said to suffer from "leukocyte adherence deficiency disease"

("LAD") (Anderson, D.C., *et al.*, *Fed. Proc.* 44:2671-2677 (1985); Anderson, D.C., *et al.*, *J. Infect. Dis.* 152:668-689 (1985)). Characteristic features of LAD patients include necrotic soft tissue lesions, impaired pus formation and wound healing, as well as abnormalities of adhesion-dependent leukocyte functions *in vitro*, and susceptibility to chronic and recurring bacterial infections. Granulocytes from these LAD patients behave in the same defective manner *in vitro* as do their normal counterparts in the presence of anti-CD18 monoclonal antibody. That is, they are unable to perform adhesion related functions such as aggregation or attachment to endothelial cells. More importantly, however, is the observation that these patients are unable to mount a normal inflammatory response because of the inability of their granulocytes to attach to cellular substrates. Most remarkable is the observation that granulocytes from these LAD patients are unable to get to sites of inflammation such as skin infections due to their inability to attach to the endothelial cells in the blood vessels near the inflammation lesions. Such attachment is a necessary step for extravasation.

Thus, in summary, the ability of lymphocytes and granulocytes to maintain the health and viability of an animal requires that they be capable of adhering to other cells (such as endothelial cells). Granulocyte-endothelial cell adherence has been found to require cell-cell contacts which involve specific receptor molecules present on the granulocyte cell surface. These receptors enable the leukocyte to adhere to other leukocytes or to endothelial, and other non-vascular cells.

The cell surface receptor molecules of leukocytes have been found to be highly related to one another. Humans whose leukocytes lack these cell surface receptor molecules exhibit chronic and recurring infections, as well as other clinical symptoms. Inflammation reactions are mitigated when leukocytes are unable to adhere in a normal fashion due to the lack of functional adhesion molecules of the CD18 complex. Because leukocyte adhesion is involved in the process through which tissue inflammation arises,

an understanding of the process of leukocyte adhesion is of significant value in defining a treatment for specific and non-specific inflammation.

Additionally, since lymphocyte adhesion is involved in the process through which foreign body or tissue is identified and rejected, an understanding of this process is of significant value in the fields of organ transplantation, tissue grafting, allergy and oncology.

C. The Intercellular Adhesion Molecule ICAM-1 and Cellular Adhesion

The intercellular adhesion molecule ICAM-1 was first identified and partially characterized according to the procedure of Rothlein, R. *et al.* (*J. Immunol.* 137:1270-1274 (1986)), which reference is herein incorporated by reference. ICAM-1, its preparation, purification, and characteristics are disclosed in WO 90/03400 which application is herein incorporated by reference in its entirety.

ICAM-1 was initially realized as being involved in the process of cellular adhesion between endothelial cells and leukocytes. Cellular adhesion is the process through which leukocytes attach to cellular substrates, such as endothelial cells, in order to migrate from circulation to sites of ongoing inflammation, and properly defend the host against foreign invaders such as bacteria or viruses. An excellent review of the defense system is provided by Eisen, H.W., (*In: Microbiology*, 3rd Ed., Harper & Row, Philadelphia, PA (1980), pp. 290-295 and 381-418).

One of the molecules on the surface of endothelial cells which participates in the adhesion process is ICAM-1. This molecule has been shown to mediate adhesion by binding to molecules of the CD-18, CD-11/18 family of glycoproteins which are present on the cell surfaces of leukocytes (Sanchez-Madrid, F. *et al.*, *J. Exper. Med.* 158:1785-1803 (1983); Keizer, G.D. *et al.*, *Eur. J. Immunol.* 15:1142-1147 (1985)).



Intercellular Adhesion Molecule (ICAM-1) is an inducible cell surface glycoprotein expressed on various cell types including vascular endothelial cells, and is expressed preferentially at sites of inflammation. Since ICAM-1 is the natural binding ligand of LFA-1, ICAM-1-LFA-1 interactions play a central role in cellular adhesion, recruitment of lymphocytes to sites of inflammation and the triggering of lymphocyte functions which contribute to both specific and non-specific inflammation.

#### D. The Cellular Receptor for Human Rhinovirus

Abraham *et al.* (*J. Virol.* 51:340-345 (1984)) discovered that the majority of randomly selected human rhinovirus ("HRV") serotypes were able to bind to the same cellular receptor. A monoclonal antibody was subsequently developed by Colonno *et al.* (Colonno *et al.*, *J. Cell. Biochem. Suppl.* 10 (part D):266 (1986); Colonno *et al.*, *J. Virol.* 57:7-12 (1986); Colonno *et al.*, European Patent Application Publication No. 169,146) which was capable of blocking attachment of HRV of the major serotype to the surfaces of endothelial cells. The endothelial cell receptor protein recognized by this antibody was isolated and found to be a 90 kd protein (Tomassini *et al.*, *J. Virol.* 58:290-295 (1986) and later shown to be the ICAM-1 molecule (Staunton *et al.*, *Cell* 56:849-854 (1989)).

Treatment of rhinoviral infection, especially infection by the major type human rhinovirus has been proposed using a murine monoclonal antibody directed against the viral receptor, ICAM-1 (EP 391088).

#### E. Infection with HIV

HIV infection is the cause of AIDS. Two major variants of HIV have been described: HIV-1 and HIV-2. HIV-1 is prevalent in North America and Europe, in contrast to HIV-2 which is prevalent only in Africa. The viruses have similar structures and encode proteins having similar function. The nucleotide and protein sequences of the genes and gene products of the two variants have been found to have about 40% homology with one another.

HIV infection is believed to occur via the binding of a viral protein (termed "gp120") to a receptor molecule (termed "CD4") present on the surface of T4 ("T helper") lymphocytes (Schnittman, S. M. *et al.*, *J. Immunol.* 141:4181-4186 (1988), which reference is incorporated herein by reference). The virus then enters the cell and proceeds to replicate, in a process which ultimately results in the death of the T cell. The destruction of an individual's T4 population is a direct result of HIV infection. HIV can be recovered from peripheral blood mononuclear cells and human plasma (*J. Clin. Microbiol.* 26:2371-2376 (1988); *N. Engl. J. Med.* 321:1621-1625 (1989)). Results suggest more viremia than had been previously estimated and a T-cell infection frequency as high as 1%.

The destruction of the T cells results in an impairment in the ability of the infected patient to combat opportunistic infections. Although individuals afflicted with AIDS often develop cancers, the relationship between these cancers and HIV infection is, in most cases, uncertain.

Although the mere replication of the HIV virus is lethal to infected cells, such replication is typically detected in only a small fraction of the T4 cells of an infected individual. Several lines of research have elucidated other mechanisms through which the HIV virus mediates the destruction of the T4 population.

Apart from through HIV replication, HIV infected cells can be destroyed through the action of cytotoxic, killer cells. Killer cells are normally present in humans, and serve to monitor the host and destroy any foreign cells (such as in mismatched blood transfusions or organ transplants, etc.) which may be encountered. Upon infection with HIV, T4 cells display the gp120 molecule on their cell surfaces. Killer cells recognize such T4 cells as foreign (rather than native cells), and accordingly, mediate their destruction.

HIV infection can also lead to the destruction of non-infected healthy cells. Infected cells can secrete the gp120 protein into the blood system. The free gp120 molecules can then bind to the CD4 receptors of healthy,

uninfected cells. Such binding causes the cells to take on the appearance of HIV infected cells. Cytotoxic, killer cells recognize the gp120 bound to the uninfected T4 cells, conclude that the cell is foreign, and mediate the destruction of the T4 cells.

5           An additional mechanism, and one of special interest to the present invention, with which HIV can cause T4 death is through the formation of "syncytia." A "syncytium" is a multinucleated giant cell, formed from the fusion of as many as several hundred T4 cells. Infection with HIV causes the infected cell to become able to fuse with other T4 cells. Such fusion partners  
10           may themselves be HIV infected, or they may be uninfected healthy cells. The syncytium cannot function and soon dies. Its death accomplishes the destruction of both HIV infected and HIV uninfected T4 cells. This process is of special interest to the present invention since it entails the direct cell-cell contact of T4 cells. The ability of HIV-infected cells to form syncytia  
15           indicates that such cells acquire a means for fusing with healthy cells. Thus, cell-cell contacts may be of fundamental importance in the process through which HIV infection is transmitted from one cell to another within an individual.

          HIV infection, and especially HIV-1 infection, appears to influence cell  
20           surface expression of the leukocyte integrins and cellular adherence reactions mediated by these heterodimers (Petit, A.J., *et al.*, *J. Clin. Invest.* 79:188 (1987); Hildreth, J.E.K., *et al.*, *Science* 244:1075 (1989); Valentin, A., *et al.*, *J. Immunology* 144:934-937 (1990); Rossen, R.D., *et al.*, *Trans. Assoc. American Physicians* 102:117-130 (1989), all of which references are  
25           incorporated herein by reference). Following infection with HIV-1, homotypic aggregation of U937 cells is increased, as is cell surface expression of CD18, CD11b (Petit, A.J., *et al.*, *J. Clin. Invest.* 79:188 (1987)). HIV-1 infected U937 cells adhere to IL-1 stimulated endothelium in greater frequency than uninfected U937 cells; this behavior can be suppressed by treating the  
30           infected cells with anti-CD18 or anti-CD11a monoclonal antibodies or by treating endothelial substrates with anti-ICAM-1 (Rossen, R.D., *et al.*, *Trans.*

*Assoc. American Physicians* 102:117-130 (1989)). Monoclonal antibodies to CD18 or CD11a have also been found to be able to inhibit formation of syncytia involving phytohemagglutinin (PHA)-stimulated lymphoblastoid cells and constitutively infected, CD4-negative T cells (Hildreth, J.E.K., *et al.*, *Science* 244:1075 (1989)). Treatment of only the virus infected cells with anti-CD18, or anti-CD11a monoclonal antibodies was found to have little effect on syncytium formation, suggesting that these antibodies principally protect uninfected target cells from infection (Hildreth, J.E.K., *et al.*, *Science* 244:1075 (1989); Valentin, A., *et al.*, *J. Immunology* 144:934-937 (1990)). Valentin *et al.* (Valentin, A., *et al.*, *J. Immunology* 144:934-937 (1990)) have recently confirmed these observations by demonstrating that monoclonal antibodies specific for CD18 inhibit syncytia formed when continuous T cell lines are co-cultured with HIV-1 infected U937 cells.

Although the mechanism through which monoclonal antibodies specific for CD18 or CD11a protect susceptible cells from fusing with HIV infected cells remains unknown, and is not necessary to an appreciation of the present invention, studies with radiolabeled gp120 suggest that heterodimers containing CD18 do not provide a binding site for the virus (Valentin, A., *et al.*, *J. Immunology* 144:934-937 (1990)). Thus, HIV infection involves cell-cell interactions, and/or viral-cell interactions which mimic such cell-cell interactions. The cell-cell interactions may result in the transport of cell-free virus or the transport of virus across endothelial barriers within the cytoplasm of infected mononuclear cells. Viral-cell interactions which mimic the cell-cell interactions may facilitate or enable free virus to attach to and/or infect healthy cells.

The present invention thus derives, in part, from the observation that HIV infection, and particularly HIV-1, infection results in increased expression of the CD11a/CD18 heterodimer, and its binding ligand, ICAM-1. This increased expression is significant in that it enhances the ability of HIV-infected T cells to adhere or aggregate with one another (i.e. to undergo "homotypic aggregation"). Since such homotypic aggregation is not observed

to occur among quiescent normal leukocytes, this discovery indicates that the expression of the CD11/CD18 receptors and/or ICAM-1 is required for such aggregation. Such adhesion permits HIV-1 to be transmitted from an infected cell to a healthy cell of an individual, and also permits or facilitates infection of healthy cells with free virus.

Since ICAM-1 plays a central role in cell-cell interactions murine monoclonal antibodies that bind to ICAM-1 have been proposed as a method of preventing HIV infection (WO 90/13281).

#### F. Migration of HIV Infected Cells

The migration and dissemination of leukocytes is important in protecting an individual from the consequences of infection. These processes, however, are also responsible for the migration and dissemination of viral-infected leukocytes. Of particular concern is the migration and dissemination of leukocytes infected with HIV. The migration of such cells results in the formation of extravascular foci, and may cause tumors and other abnormalities.

Histologic examination of affected organs reveals focal extravascular mononuclear cell infiltrates. Attempts to identify virus-infected cells in such infiltrates in the central nervous system have revealed the presence of HIV-1 infected cells. These studies have shown that HIV-1 resides primarily in monocytes and macrophages, and other cells of this lineage (R.T. Johnson, *et al. FASEB J.* 2:2970 (1988); M.H. Stoler *et al., J. Amer. Med. Assn.* 256:2360 (1986); S. Gartner *et al. J. Amer. Med. Assn.* 256:2365 (1986); S. Gartner *et al. Science* 233:215 (1986)).

The mechanisms which stimulate formation of extravascular infiltrates of HIV-1-infected monocytoïd cells have not previously been well defined. The mechanisms may involve either the transport of cell-free virus or the transport of virus across endothelial barriers within the cytoplasm of infected mononuclear cells.

Since infection with HIV-1 stimulates cell surface expression of molecules which facilitate adherence of leukocytes to vascular endothelial cells and the translocation of leukocytes from the blood to extravascular tissue sites (C.W. Smith *et al.*, *J. Clin. Invest.* 82:1746 (1988), herein incorporated by reference) it has been proposed to use antibodies which inhibit cellular migration to prevent the dissemination of HIV infected cells (WO 90/13316).

#### G. Asthma: Clinical Characteristics

Asthma is a heterogeneous family of diseases. It is characterized by a hyper-responsiveness of the tracheobronchi to stimuli (McFadden, E.R. *et al.*, In: *Harrison's Principles of Internal Medicine*, 10th Ed., Petersdorf, R.G. *et al.*, Eds., McGraw-Hill, NY (1983), pages 1512-1519); Kay, A.B., *Allergy and Inflammation*, Academic Press, NY (1987); which references are incorporated herein by reference). Clinically, asthma is manifested by the extensive narrowing of the tracheobronchi, by thick tenacious secretions, by paroxysms of dyspnea, cough, and wheezing. Although the relative contribution of each of these conditions is unknown, the net result is an increase in airway resistance, hyperinflation of the lungs and thorax, abnormal distribution of ventilation and pulmonary blood flow. The disease is manifested in episodic periods of acute symptoms interspersed between symptom-free periods. The acute episodes result in hypoxia, and can be fatal. Approximately 3% of the general world population suffers from the disease.

Two types of asthma have been described: allergic asthma and idiosyncratic asthma. Allergic asthma is usually associated with a heritable allergic disease, such as rhinitis, urticaria, eczema, etc. The condition is characterized by positive wheal-and-flare reactions to intradermal injections of airborne antigens (such as pollen, environmental or occupational pollutants, etc.), and increased serum levels of IgE. The development of allergic asthma appears to be causally related to the presence of IgE antibodies in many patients. Asthma patients who do not exhibit the above-described characteristics are considered to have idi syncratic asthma.

Allergic asthma is believed to be dependent upon an IgE response controlled by T and B lymphocytes and activated by the interaction of airborne antigen with mast cell-bound pre-formed IgE molecules. The antigenic encounter must occur at concentrations sufficient to lead to IgE production for  
5 a prolonged period of time in order to sensitize an individual. Once sensitized, an asthma patient may exhibit symptoms in response to extremely low levels of antigen.

Asthma symptoms may be exacerbated by the presence and level of the triggering antigen, environmental factors, occupational factors, physical  
10 exertion, and emotional stress.

Asthma may be treated with methylxanthines (such as theophylline), beta-adrenergic agonists (such as catecholamines, resorcinols, saligenins, and ephedrine), glucocorticoids (such as hydrocortisone), inhibitors of mast cell degranulation (i.e. chromones such as cromolyn sodium) and anticholinergics  
15 (such as atropine).

Asthma is believed to involve an influx of eosinophils ("eosinophilia") into the tissues of the lung (Frigas, E. *et al.*, *J. Allergy Clin. Immunol.* 77:527-537 (1986), which reference is incorporated herein by reference).

Insight into the immunological basis of asthma has been gained from bronchoalveolar lavage studies (Godard, P. *et al.*, *J. Allergy Clin. Immunol.* 70:88 (1982)), and studies of respiratory smooth muscle denuded of epithelium (Flavahan, N.A. *et al.*, *J. Appl. Physiol.* 58:834 (1985); Barnes, P. J. *et al.*, *Br. J. Pharmacol.* 86:685 (1985)). Although these studies have not led to the elucidation of the mechanism underlying the immunology of  
20 asthma, they have led to the development of a generally accepted hypothesis concerning the immunological etiology of the disease (see, Frigas, E. *et al.*, *J. Allergy Clin. Immunol.* 77:527-537 (1986)).  
25

The hallmarks of the pathology of asthma are a massive infiltration of the lung parenchyma by eosinophils and the destruction of mucociliary capacity. The "eosinophil hypothesis" suggests that eosinophils are attracted  
30 to the bronchus in order to neutralize harmful mediators released by the mast

cells of the lung. According to the hypothesis eosinophils are attracted to the bronchi where they degranulate to release cytotoxic molecules. Upon degranulation, eosinophils release enzymes such as histaminase, arylsulfatase and phospholipase D which enzymatically neutralize the harmful mediators of the mast cell. These molecules also promote the destruction of the mucociliary apparatus, and thus prevent the clearing of the bronchial secretions, and contribute to the lung damage characteristic of asthma.

Since asthma involves the migration of cells, it has been proposed to use antibodies which inhibit this migration to mitigate the effects of allergens in a subject (WO 90/10453).

#### H. Conclusion

It has been previously proposed; to treat leucocyte-mediated inflammation by administering *inter alia* an anti-ICAM-1 antibody to patients suffering from such inflammation (see EP-0289949 and EP-0314863), to treat viral infection by administering *inter alia* an anti-ICAM-1 antibody to patients suffering from such infection (EP 391088), to prevent the infection of a subject with HIV by administering *inter alia* an anti-ICAM-1 antibody (WO 90/13281), to prevent the dissemination of HIV infected cells by administering *inter alia* an anti-ICAM-1 antibody (WO 90/13316), and to administer *inter alia* an anti-ICAM-1 antibody to mitigate the effects of allergens (WO 90/10453).

EP 289949 describes the preparation of a murine monoclonal (R6-5-D6) having specificity for ICAM-1 which is the preferred antibody for the above referenced therapies. Samples of R6-5-D6 have been deposited with the American Type Culture Collection as deposit ATCC HB9580 on 30th October 1987. R6-5-D6 has been deposited with the ATCC under the provisions of Rule 28(4) of the EPC.

Currently available anti-ICAM-1 MAbs, which are the basis of the above described methods of treatment, are murine MAbs and as a result are



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likely to cause a significant HAMA response if administered in repeat doses to human patients. It would be highly desirable to diminish or abolish this undesirable HAMA response by suitable humanization or other appropriate recombinant DNA manipulation of these potentially highly useful antibodies and thus extend and enlarge their use. It would also be desirable to apply the techniques of recombinant DNA technology to these antibodies to prepare anti-ICAM-1 humanized chimeric antibodies in general.

We have now prepared anti-ICAM-1 chimeric humanized antibody molecules derived from murine MAbs.

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#### SUMMARY OF THE INVENTION

The present invention provides a method of constructing a chimeric antibody molecule. Specifically the present invention provides a chimeric antibody molecule comprising heavy and/or light chain variable regions of an anti-ICAM-1 antibody.

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The invention further pertains to the chimeric antibody of the present invention which are detectably labeled.

The present invention further provides a process for producing an anti-ICAM-1 humanized chimeric antibody molecule.

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The present invention further provides DNA coding for a heavy or light chain variable region of a chimeric anti-ICAM-1 antibody.

The invention additionally includes a recombinant DNA molecule capable of expressing the chimeric antibodies of the present invention.

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The invention further includes a host cell capable of producing the chimeric antibodies of the present invention when transformed by the recombinant DNA molecules disclosed herein.

The invention additionally includes diagnostic and therapeutic uses for the chimeric antibodies of the present invention.

The invention further provides a method for treating inflammation resulting from a response of the specific defense system in a mammalian

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subject which comprises providing to a subject in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress the inflammation, wherein the anti-inflammatory agent is a humanized chimeric antibody capable of binding to ICAM-1.

5           The invention further provides a method for treating non-specific inflammation in humans, and other mammals.

          In detail, the invention includes a method for treating inflammation resulting from a response of the specific and non-specific defense system in a mammalian subject which comprises providing to a subject in need of such  
10          treatment an anti-inflammatory agent, capable of binding to an ICAM-1, in an amount sufficient to suppress the inflammation; wherein the anti-inflammatory agent is a humanized chimeric antibody capable of binding to ICAM-1.

          The invention further includes the above-described method for treating inflammation wherein the inflammation is associated with a condition selected  
15          from the group consisting of: adult respiratory distress syndrome; multiple organ injury syndrome secondary to septicemia; multiple organ injury syndrome secondary to trauma; reperfusion injury of myocardial or other tissues; acute glomerulonephritis; reactive arthritis; dermatosis with acute inflammatory components; acute purulent meningitis or other central nervous  
20          system inflammatory disorders such as stroke; thermal injury; hemodialysis; leukapheresis; ulcerative colitis; Crohn's disease; necrotizing enterocolitis; granulocyte transfusion associated syndrome; and cytokine-induced toxicity.

          The invention further provides a method of suppressing the metastasis of a hematopoietic tumor cell, the cell requiring a functional member of the  
25          LFA-1 family for migration, wherein said method comprises providing to a patient in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress the metastasis; wherein the anti-inflammatory agent is a humanized chimeric antibody capable of binding to ICAM-1.

          The invention further provides a method of suppressing the growth of  
30          an ICAM-1-expressing tumor cell which comprises providing to a patient in need of such treatment an amount of a toxin sufficient to suppress the growth,

the toxin being derivatized to one of the chimeric antibodies of the present invention.

The invention further provides a method of diagnosing the presence and location of an inflammation resulting from a response of the specific defense system in a mammalian subject suspected of having the inflammation which comprises:

(a) administering to the subject a composition containing a detectably labeled chimeric antibody capable of identifying a cell which expresses ICAM-1, and

(b) detecting the binding ligand.

The invention additionally provides a method of diagnosing the presence and location of an inflammation resulting from a response of the specific defense system in a mammalian subject suspected of having the inflammation which comprises:

(a) incubating a sample of tissue of the subject with a composition containing a detectably labeled chimeric antibody capable of identifying a cell which expresses ICAM-1, and

(b) detecting the binding ligand.

The invention further provides a method of diagnosing the presence and location of an ICAM-1-expressing tumor cell in a mammalian subject suspected of having such a cell, which comprises:

(a) administering to the subject a composition containing a detectably labeled chimeric antibody capable of binding to ICAM-1, and

(b) detecting the binding ligand.

The invention further provides a method of diagnosing the presence and location of an ICAM-1-expressing tumor cell in a mammalian subject suspected of having such a cell, which comprises:

(a) incubating a sample of tissue of the subject with a composition containing a detectably labeled chimeric antibody capable of binding ICAM-1, and

(b) detecting the binding ligand.

The invention additionally includes a pharmaceutical composition comprising:

(a) an anti-inflammatory agent consisting of a chimeric antibody capable of binding to ICAM-1, and

5 (b) at least one immunosuppressive agent selected from the group consisting of: dexamethasone, azathioprine and cyclosporin A.

The present invention also relates to the use of chimeric antibodies capable of binding ICAM-1 in anti-viral therapy.

10 In detail, the invention provides a method for treating viral infection, wherein said virus binds to the ICAM-1 receptor, in an individual in need of such treatment, wherein the method comprises providing to the individual an amount of a chimeric antibody capable of binding ICAM-1 sufficient to suppress viral infection.

15 The invention further provides a method for suppressing the infection of leukocytes with HIV, which comprises administering to a patient exposed to or effected by HIV, an effective amount of an HIV-1 infection suppression agent, the agent being a chimeric antibody capable of binding to ICAM-1.

The invention further concerns the embodiment of the above method wherein the HIV is HIV-1.

20 The invention further provides a method for suppressing the extravascular migration of a virally infected leukocyte in a patient having such a leukocyte, which comprises administering to the patient an effective amount of a chimeric antibody capable of impairing the ability of said leukocyte to bind to ICAM-1.

25 The invention further comprises the embodiment of the above-described method wherein the virally infected leukocytes are infected with HIV.

The invention further comprises the embodiment of the above-described method wherein the agent is a chimeric antibody capable of binding to ICAM-1.

The invention further provides a method for treating asthma in a patient which comprises providing to the patient an effective therapeutic amount of a chimeric antibody capable of binding to ICAM-1

5 The invention further concerns the embodiment of the above described methods wherein the chimeric antibody capable of binding to ICAM-1 is derived from the murine monoclonal antibody R6-5-6D.

Brief Description of the Figures

- 10 Figure 1 shows the cDNA sequence for the 5' untranslated region, signal sequence, variable region and part constant region for the R6-5-D6 murine MAb light chain;
- Figure 2 shows similar cDNA and amino acid sequence for the R6-5-D6 murine MAb heavy chain;
- Figure 3 shows a plasmid diagram of plasmid expression vector pEE6 hCMV;
- 15 Figure 4 shows plasmid diagrams indicating the strategy for construction of light chain expression plasmid pAL5;
- Figure 5 shows plasmid diagrams indicating the strategy for construction of heavy chain expression plasmid pAL6;
- 20 Figure 6 shows a graph giving results of a competition assay comprising binding of recombinant and murine R6-5-D6 and a control MAb UPC10;
- Figure 7 shows plasmid diagrams indicating the strategy for the construction of chimeric light chain expression vector pAL7;
- Figure 8 shows plasmid diagrams indicating the strategy for the construction of chimeric heavy chain (IgG2 isotype) expression vector pAL8;
- 25 Figure 9 shows outline restriction maps for the chimeric heavy chain expression vectors pAL8 and pAL9;

- Figure 10 shows plasmid diagrams indicating the procedures involved in the construction of the GS amplification chimeric light chain expression vector pAL10;
- 5 Figure 11 shows similar plasmid diagrams for the construction of the GS chimeric heavy chain (IgG2 isotype) expression vector pAL12;
- Figure 12 shows similar diagrams for the chimeric heavy chain (IgG4 isotype);
- Figure 13 shows SDS-PAGE analysis under non-reducing and reducing conditions. The notation above each lane describes the type of gene used in the transient expression experiment.
- 10
- |                                      |                           |
|--------------------------------------|---------------------------|
| mL mouse light                       | cL chimeric light         |
| $\gamma_4$ chimeric $\gamma_4$ heavy | mH mouse heavy            |
| $\gamma_2$ chimeric $\gamma_2$ heavy | B72.3 control cL/cH genes |
- Figure 14 shows SDS-PAGE analysis of purified chimeric anti-ICAM-1 antibody or (A) non-reducing and (b) reducing gels.
- 15
- On each gel Lane 1 is control chimeric B72.3 antibody (IgG4)
- Lane 2 is Pharmacia low molecular weight markers
- 20 Lanes 3-9 are chimeric anti-ICAM IgG2  $4\mu\text{g} \rightarrow 0.125\mu\text{g}$  in doubling dilutions
- Lane 10 is Pharmacia low molecular weight markers
- 25 Lanes 11-17 are chimeric anti-ICAM IgG4  $4\mu\text{g} \rightarrow 0.125\mu\text{g}$  in doubling dilutions
- Figure 15 shows HPLC Gel filtration of chimeric anti-ICAM antibody of IgG2 and IgG4 isotypes.
- 30 The profiles are superimposable and elute at a time which corresponds to 150kd tetrameric antibody.

- Figures 16 shows graphs of binding assays of chimeric  
and 17 antibodies against standards, and  
Figures 18 shows graphs of competition binding assays  
19 and 20 of chimeric antibodies against standards.  
5 Figure 21 Inhibition of MLR with antibodies.  
Figure 22 Inhibition of Vascular permeability in a modified  
Schwartzmann Assay with antibodies.

### **Brief Description of the Preferred Embodiments**

#### **A. Humanized Antibodies**

- 10 The first embodiment of the present invention provides a chimeric  
antibodies molecule comprising heavy and/or light chain variable regions of  
an anti-ICAM-1 antibody.

Typically the anti-ICAM-1 antibody is a rodent MAb.

- Specifically, in the first embodiment of the present invention the  
15 chimeric antibody is a humanized chimeric antibody molecule. In which case  
the chimeric antibody comprises heavy and/or light chain variable regions of  
a non-human (e.g., rodent) anti-ICAM-1 antibody linked to heavy and/or light  
chain constant region domains of a human antibody. The DNA which codes  
for such humanized chimeric heavy and/or light chains comprises DNA coding  
20 for the non-human variable region domains linked to DNA coding for human  
constant region domains.

- The chimeric antibodies molecule of the present invention may  
comprise: a complete antibody molecule, having full length heavy and light  
chains; a fragment thereof, such as the Fab or (Fab')<sub>2</sub> fragment; a light chain  
25 or heavy chain monomer or dimer, including fragments thereof or any  
chimeric antibody molecule with the same specificity as an anti-ICAM-1  
antibody.

The chimeric antibodies of the present invention may be a "chemical  
derivative" of the antibody. As used herein, a molecule is said to be a

"chemical derivative" of another molecule when it contains additional chemical moieties not normally a part of the molecule. Such moieties may improve the molecule's solubility, absorption, biological half life, etc. The moieties may alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, etc. Moieties capable of mediating such effects are disclosed in *Remington's Pharmaceutical Sciences* (1980). "Toxin-derivatized" molecules constitute a special class of "chemical derivatives." A "toxin-derivatized" molecule is a molecule (such as ICAM-1 or an antibody) which contains a toxin moiety. The binding of such a molecule to a cell brings the toxin moiety into close proximity with the cell and thereby promotes cell death. Any suitable toxin moiety may be employed; however, it is preferable to employ toxins such as, for example, the ricin toxin, the diphtheria toxin, radioisotopic toxins, membrane-channel-forming toxins, etc. Procedures for coupling such moieties to a molecule are well known in the art. Alternatively the chimeric antibody can be attached to a macrocycle, for chelating a heavy metal atom.

Alternatively, the procedures of recombinant DNA technology may be used to produce a "chemical derivative" of the chimeric antibody in which the Fc fragment or CH3 domain of a complete antibody molecule has been replaced by or has attached thereto by peptide linkage a functional non-immunoglobulin protein such as an enzyme or toxin molecule.

In the case of humanized chimeric antibody molecules, the remainder of the molecule may be derived from any suitable human immunoglobulin. Human constant region domains may be selected having regard to the proposed function of the antibody in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgE, IgG or IgM domains. In particular, IgG human constant region domains may be used including any of the IgG1, IgG2, IgG3 and IgG4 isotypes. Thus IgG2 or preferably IgG4 isotypes may be used when the humanized chimeric antibody is intended for therapeutic purposes, requiring an absence of antibody effector functions e.g., to block ICAM-1-LFA-1



interactions. Chimeric IgG anti-ICAM-1 antibodies may have different avidities depending on isotype and this may influence the therapeutic choice. Most preferably IgG1 human constant region domains are used. We have found that a IgG1 chimeric antibody appears to have a higher binding avidity for ICAM-1 than IgG2 or IgG4 chimeric antibodies, possibly due to a greater flexibility of the IgG1 hinge promoting bivalent binding to the antigen.

The remainder of the humanized chimeric antibody molecule need not comprise only protein sequences from the human immunoglobulin. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequence of a polypeptide effector or reporter molecule.

Preferably, the chimeric antibody molecule of the present invention will be produced by recombinant DNA technology.

A second embodiment of the present invention provides DNA coding for a heavy or light chain variable region of an anti-ICAM-1 antibody.

A third embodiment of the present invention provides a process for producing an anti-ICAM-1 humanized chimeric antibody molecule which process comprises:

- (a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy or light chain wherein at least the variable domain is derived from a non-human (rodent) anti-ICAM-1 antibody and the remaining immunoglobulin-derived parts of the antibody chain are derived from a human immunoglobulin;
- (b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light or heavy chain wherein at least the variable domain is derived from a non-human (rodent) anti-ICAM-1 antibody and the remaining immunoglobulin-derived parts of the antibody chain are derived from a human immunoglobulin;

- (c) transfecting a host cell with the or each vector; and
- (d) culturing the transfected cell line to produce the humanized chimeric antibody molecule.

5 The cell line may be transfected with two vectors, the first vector containing an operon encoding a light chain-derived polypeptide and the second vector containing an operon encoding a heavy chain-derived polypeptide. Preferably, the vectors are identical except in so far as the coding sequences and selectable markers are concerned so as to ensure as far as possible that each polypeptide chain is equally expressed.

10 Alternatively, a single vector may be used, the vector including the sequences encoding both light chain- and heavy chain-derived polypeptides.

The DNA in the coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both. However, it is preferred that the DNA sequence encoding the heavy or light chain comprises at least partially  
15 genomic DNA. Most preferably, the heavy or light chain encoding sequence comprises a fusion of cDNA and genomic DNA.

Thus, the present invention also includes cloning and expression vectors and transfected cell lines used in the process of the invention.

20 The general methods by which the vectors may be constructed, transfection methods and culture methods are well known *per se* and form no part of the invention. Such methods are shown, for instance, in Maniatis *et al.*, *Molecular Cloning*, Cold Spring Harbor, New York (1982); and Primrose and Old, *Principles of Gene Manipulation*, Blackwell, Oxford (1980).

25 The anti-ICAM-1 antibodies of the invention include all anti-ICAM-1 specificities. Typically, however, the antibodies have specificity for antigenic epitopes of ICAM-1 which when bound by the antibody block, inhibit or otherwise modify ICAM-1/LFA-1 and or ICAM-1/Mac-1 interactions. Preferably the antibodies have specificity for the same or similar ICAM-1

antigenic epitopes as the R6-5-D6 etc. antibodies. Most especially the antibodies are derived from the R6-5-D6 antibody.

## B. Therapeutics and Diagnosis

5 The present invention also includes therapeutic and diagnostic compositions containing the chimeric antibodies of the invention and uses of such compositions in therapy and diagnosis.

The therapeutic uses to which the products of the anti-ICAM-1 invention may be put include any of the therapeutic uses to which anti-ICAM-1 antibodies may be put including for example any or all of the therapeutic  
10 uses described in EP-0289949, EP-0314863, and corresponding applications.

### 1. Anti-Inflammatory Agents

#### A. Specific Inflammation

Monoclonal antibodies to members of the CD 18 or CD-11/18 complex inhibit many adhesion dependent functions of leukocytes including binding to  
15 endothelium (Haskard, D., *et al.*, *J. Immunol.* 137:2901-2906 (1986)), homotypic adhesions (Rothlein, R., *et al.*, *J. Exp. Med.* 163:1132-1149 (1986)), antigen and mitogen induced proliferation of lymphocytes (Davignon, D., *et al.*, *Proc. Natl. Acad. Sci., USA* 78:4535-4539 (1981)), antibody formation (Fischer, A., *et al.*, *J. Immunol.* 136:3198-3203 (1986)), and  
20 effector functions of all leukocytes such as lytic activity of cytotoxic T cells (Krensky, A.M., *et al.*, *J. Immunol.* 132:2180-2182 (1984)), macrophages (Strassman, G., *et al.*, *J. Immunol.* 136:4328-4333 (1986)), and all cells involved in antibody-dependent cellular cytotoxicity reactions (Kohl, S., *et al.*, *J. Immunol.* 133:2972-2978 (1984)). In all of the above functions, the  
25 antibodies inhibit the ability of the leukocyte to adhere to the appropriate

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cellular substrate which in-turn-inhibits the final outcome. Although both polyclonal and monoclonal antibodies may be employed to inhibit these function, the present invention provides an improvement through the use of a chimeric anti-ICAM-1 antibody.

5           As discussed previously, the binding of ICAM-1 molecules to the members of LFA-1 family of molecules is of central importance in cellular adhesion. Through the process of adhesion, lymphocytes are capable of continually monitoring an animal for the presence of foreign antigens. Although these processes are normally desirable, they are also the cause of  
10 organ transplant rejection, tissue graft rejection and many autoimmune diseases. Hence, any means capable of attenuating or inhibiting cellular adhesion would be highly desirable in recipients of organ transplants, (e.g., kidney), tissue grafts or autoimmune patients.

          A chimeric antibody capable of binding to ICAM-1 is highly suitable  
15 as an anti-inflammatory agent in a mammalian subject. Significantly, such an agent differs from general anti-inflammatory agents and non-humanized antibodies in that they are capable of selectively inhibiting adhesion, do not offer other side effects such as nephrotoxicity which are found with conventional agents, and limit the amount of HAMA associated with the use of  
20 murine MAbs. A chimeric antibody capable of binding to ICAM-1 can therefore be used to prevent organ (e.g. kidney) or tissue rejection, or modify autoimmune responses without the fear of such side effects, in the mammalian subject.

          Importantly, the use of humanized antibodies capable of recognizing  
25 ICAM-1 may permit one to perform organ transplants even between individuals having HLA mismatch.

          In the forth embodiment of the present invention a method for suppressing specific inflammation is provided wherein said method comprises providing to recipient subjects in need of such a treatment an amount of one  
30 of the chimeric antibodies of the present invention sufficient to suppress inflammation. An amount is said to be sufficient to "suppress" inflammation

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if the dosage, route of administration, etc. of the agent are sufficient to attenuate or prevent inflammation.

5 The chimeric antibody may be administered either alone or in combination with one or more additional immunosuppressive agents (especially to a recipient of an organ (e.g. kidney) or tissue transplant). The administration of such a composition may be for either a "prophylactic" or "therapeutic" purpose. When provided prophylactically, the immunosuppressive composition is provided in advance of any inflammatory response or symptom (for example, prior to, at, or shortly after) the time of  
10 an organ or tissue transplant but in advance of any symptoms of organ rejection). The prophylactic administration of the composition serves to prevent or attenuate any subsequent inflammatory response (such as, for example, rejection of a transplanted organ or tissue, etc.). When provided therapeutically, the immunosuppressive composition is provided at (or shortly  
15 after) the onset of a symptom of actual inflammation (such as, for example, organ or tissue rejection). The therapeutic administration of the composition serves to attenuate any actual inflammation (such as, for example, the rejection of a transplanted organ or tissue).

20 The anti-inflammatory agents of the present invention may, thus, be provided either prior to the onset of inflammation (so as to suppress an anticipated inflammation) or after the initiation of inflammation.

Since ICAM-1 molecules are expressed mostly at sites of inflammation, such as those sites involved in delayed type hypersensitivity reaction, antibodies (especially chimeric antibodies derived from anti-ICAM-1  
25 monoclonal antibodies) capable of binding to ICAM-1 molecules have therapeutic potential in the attenuation or elimination of such reactions. This potential therapeutic use may be exploited in either of two manners. First, a composition containing a chimeric antibody capable of binding to ICAM-1 may be administered to a patient experiencing delayed type hypersensitivity  
30 reaction. For example, such compositions might be provided to a individual who had been in contact with antigens such as poison ivy, poison oak, etc.

In a sixth embodiment, one of the chimeric antibodies of the present invention is administered to a patient in conjunction with an antigen in order to prevent a subsequent inflammatory reaction. Thus, the additional administration of an antigen with an ICAM-1-binding chimeric antibody may temporarily tolerize an individual to subsequent presentation of that antigen.

Since LAD patients that lack LFA-1 do not mount an inflammatory response, it is believed that antagonism of LFA-1's natural ligand, ICAM-1, will also inhibit an inflammatory response. The ability of antibodies against ICAM-1 to inhibit inflammation provides the basis for their therapeutic use in the treatment of chronic inflammatory diseases and autoimmune diseases such as lupus erythematosus, autoimmune thyroiditis, experimental allergic encephalomyelitis (EAE), multiple sclerosis, some forms of diabetes Reynaud's syndrome, rheumatoid arthritis, etc. Such antibodies may also be employed as a therapy in the treatment of psoriasis. In general, a chimeric antibody capable of binding ICAM-1 may be employed in the treatment of those diseases currently treatable through steroid therapy.

#### B. Non-specific Inflammation

The present invention derives from the discovery that ICAM-1 on endothelial cells binds to the members of the CD18 family of molecules on granulocytes responsible for mediating granulocyte-endothelial cell adhesion and that antagonists of ICAM-1 are capable of inhibiting such adhesion. Such inhibition provides a means for treating general, non-specific tissue inflammation.

Since cellular adhesion is required in order that leukocytes may migrate to sites of non-specific inflammation and/or carry out various effector functions contributing to the inflammation, agents which inhibit cellular adhesion will attenuate or prevent this inflammation. A "non-specific defense system reaction" is a response mediated by leukocytes incapable of immunological memory. Such cells include granulocytes and macrophages. As used herein, inflammation is said to result from a response of the non-

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specific defense system, if the inflammation is caused by, mediated by, or associated with a reaction of the non-specific defense system. Examples of inflammation which result, at least in part, from a reaction of the non-specific defense system include inflammation associated with conditions such as: adult  
5 respiratory distress syndrome (ARDS) or multiple organ injury syndromes secondary to septicemia or trauma; reperfusion injury of myocardial or other tissues; acute glomerulonephritis; reactive arthritis; dermatoses with acute inflammatory components; acute purulent meningitis or other central nervous system inflammatory disorders, e.g. stroke; thermal injury; hemodialysis;  
10 leukapheresis; ulcerative colitis; Crohn's disease; necrotizing enterocolitis; granulocyte transfusion associated syndromes; and cytokine-induced toxicity.

In a fifth embodiment of the present invention a method of treating non-specific inflammation is provided wherein said method comprises providing to a subject in need of such treatment an effective amount of one of  
15 the chimeric antibody of the present invention.

## 2. Diagnostic and Prognostic Applications

Since ICAM-1 is expressed mostly at sites of inflammation, a chimeric antibody capable of binding ICAM-1 may be employed as a means of imaging or visualizing the sites of infection and inflammation in a patient.

20 In an eighth embodiment of the present invention, a chimeric antibody is detectably labeled, through the use of radioisotopes, affinity labels (such as biotin, avidin, etc.) fluorescent labels, paramagnetic atoms, etc and are provided to a patient to localize the site of infection or inflammation. Procedures for accomplishing such labeling are well known to the art. Clinical  
25 application of antibodies in diagnostic imaging are reviewed by Grossman, H.B., *Urol. Clin. North Amer.* 13:465-474 (1986)), Unger, E.C. *et al.*, *Invest. Radiol.* 20:693-700 (1985)), and Khaw, B.A. *et al.*, *Science* 209:295-297 (1980)).

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5 The detection of foci of such detectably labeled antibodies is indicative of a site of inflammation or tumor development. In one embodiment, this examination for inflammation is done by removing samples of tissue, including blood cells, and incubating such samples in the presence of the detectably labeled antibodies. In a preferred embodiment, this technique is done in a non-invasive manner through the use of magnetic imaging, fluorography, etc. Such a diagnostic test may be employed in monitoring organ transplant recipients for early signs of potential tissue rejection. Such assays may also be conducted in efforts to determine an individual's predilection to rheumatoid arthritis or other chronic inflammatory diseases.

3. Adjunct to the Introduction of Antigenic Material Administered for Therapeutic or Diagnostic Purposes

15 Immune responses to therapeutic or diagnostic agents such as, for example, bovine insulin, interferon, tissue-type plasminogen activator or murine monoclonal antibodies substantially impair the therapeutic or diagnostic value of such agents, and can, in fact, causes diseases such as serum sickness. Such a situation can be remedied through the use of the chimeric antibodies of the present invention. In this embodiment, such antibodies would be administered in combination with the therapeutic or diagnostic agent.

20 In a ninth embodiment of the present invention the addition of an effective amount of a chimeric antibody with specificity to ICAM-1 is administer to a subject in order to prevent the recipient from recognizing the agent, and therefore prevent the recipient from initiating an immune response against it. The absence of such an immune response results in the ability of the patient to receive additional administrations of the therapeutic or diagnostic agent.

4. Anti-viral usage of chimeric antibodies



Another aspect of the present invention relates to the discovery of that ICAM-1 is the cellular receptor of certain viruses, and is thus required in order for the virus to adhere to and infect human cells (Greve, J.M. *et al.*, *Cell* 56:839-847 (1989); Staunton, D.E. *et al.*, *Cell* 56:849-853 (1989), both of which references are incorporated by reference herein in their entirety). In particular, rhinoviruses, and especially rhinoviruses of the major serotype have been found to be capable of mediating their infection through their capacity to bind to the ICAM-1 molecules present on cell surfaces.

The tenth embodiment of the present invention is directed toward the use of chimeric antibodies capable of binding ICAM-1 to treat viral infection. Because such antibodies are capable of blocking the ICAM-1 of endothelial cells for viral attachment, their administration to a recipient individual results in the decrease in receptors available for viral binding, and thus decreases the percentage of viruses which attach and infect the cells of an individual.

ICAM-1 has the ability to interact with and bind to viruses, and in particular, rhinoviruses of the major serotype within the genus Picornaviridae, group A coxsackieviruses (Colonno, R.J. *et al.*, *J. virol.* 57:7-12 (1986)) and Mengo viruses (Rossmann, M.G. *et al.*, *Viol.* 164:373-382 (1988)). This interaction is mediated by ICAM-1 amino acid residues which are present in domain 1 of the ICAM-1 molecule. Such interactions are assisted, however, by contributions from amino acids present in domains 2 and 3 of ICAM-1. Thus, among the preferred chimeric antibodies of this embodiment are antibodies capable of binding to domains 1, 2, and 3 of ICAM-1. More preferred are chimeric antibodies capable of binding to domains 1 and 2 of ICAM-1. Most preferred are chimeric antibodies capable of binding domain 1 of ICAM-1.

The administration of the anti-viral agents of the present invention may be for either a "prophylactic" or "therapeutic" purpose. When provided prophylactically, the anti-viral agent is provided in advance of any symptom of viral infection (for example, prior to, at, or shortly after the time of

infection, but in advance of any symptoms of such infection). The prophylactic administration of the agent serves to prevent or attenuate any subsequent viral infection, or to reduce the possibility that such infection will be contagious to others.

5           When provided therapeutically, the anti-viral agent is provided at (or shortly after) the onset of a symptom of actual viral infection (such as, for example, nasal congestion, fever, etc. The therapeutic administration of the agent serves to attenuate any actual viral infection.

10           The anti-viral agents of the present invention may, thus, be provided either prior to the onset of viral infection (so as to suppress an anticipated infection) or after the initiation of such infection.

5.   Suppression of HIV Infection and the prevention of the Dissemination of HIV Infected Cells.

15           The eleventh embodiment of present invention provides a method for suppressing the infection of HIV, which comprises administering to an HIV-infected individual an effective amount of an HIV infection suppression agent. Although the invention is particularly concerned with a method for the suppression of HIV-1 infection, it is to be understood that the method may be applied to any HIV-1 variant (such as, for example, HIV-2) which may infect  
20           cells in a way which may be suppressed by the agents of the present invention. Such variants are the equivalents of HIV-1 for the purposes of the present invention.

25           One aspect of the present invention derives from the recognition that expression of LFA-1 and, in some cases, ICAM-1, stimulated by HIV infection, promotes cell-to-cell adherence reactions that can increase the contact time of infected with uninfected cells, facilitating transfer of virus from infected to uninfected cells. Thus, chimeric antibodies capable of binding ICAM-1 are able to suppress infection by HIV, and, in particular, by HIV-1.

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One means through which molecules which bind to ICAM-1 may suppress HIV infection is by impairing the ability of the ICAM-1 expressed by HIV-infected cells to bind to the CD11/CD18 receptors of a healthy T cell. In order to impair the ability of a cell to bind to the CD11a/CD18 receptor, or to the ICAM-1 ligand molecule, it is possible to employ chimeric antibodies capable of binding to ICAM-1.

The agents of the present invention are intended to be provided to recipient subjects in an amount sufficient to achieve a suppression of HIV infection. An amount is said to be sufficient to "suppress" HIV infection if the dosage, route of administration, etc. of the agent are sufficient to attenuate or prevent such HIV infection. The agents are to be provided to patients who are exposed to, or effected by HIV infection.

The chimeric antibodies of the present invention may be for either a "prophylactic" or "therapeutic" purpose in the treatment of HIV infection. When provided prophylactically, the antibody is provided in advance of any symptom of viral infection (for example, prior to, at, or shortly after) the time of such infection, but in advance of any symptoms of such infection). The prophylactic administration of the antibody serves to prevent or attenuate any subsequent HIV infection. When provided therapeutically, the antibody is provided at (or shortly after) the detection of virally infected cells. The therapeutic administration of the antibody serves to attenuate any additional HIV infection.

The agents of the present invention may, thus, be provided either prior to the onset of viral infection (so as to suppress the anticipated HIV infection) or after the actual detection of such virally infected cells (to suppress further infection).

In particular, the invention provides an improved therapy for AIDS, and an enhanced means for suppressing HIV infection, and particularly HIV-1 infection, which comprises the co-administration of:

- 5 (I) ICAM-1, a soluble ICAM-1 derivative, CD11 (either CD11a, CD11b, or CD11c), a soluble CD11 derivative, CD18, a soluble CD18 derivative, or a CD11/CD18 heterodimer, or a soluble derivative of a CD11/CD18 heterodimer and/or
- (II) a chimeric antibody capable of binding to ICAM-1 with
- 10 (III) cell or particle associated CD4 or a soluble derivative of CD4 and/or
- (IV) a molecule (preferably an antibody or antibody fragment) capable of binding to CD4.

15 In the twelfth embodiment of the present invention also a method for suppressing the migration of HIV-infected cells is provided wherein said method comprises administering an effective amount of an anti-migration agent to an HIV-infected individual.

20 The anti-migration agents of the present invention include any chimeric antibody capable of impairing the ability of an HIV-infected T cell to bind to ICAM-1. Chimeric antibodies which bind to ICAM-1 will suppress migration by impairing the ability of the ICAM-1 expressed by HIV-infected T cells to bind to cells expressing a CD11/CD18 receptor. In order to impair the ability of a cell to bind to the CD11a/CD18 receptor it is possible to employ a chimeric antibody capable of binding to ICAM-1.

25 The agents of the present invention are intended to be provided to recipient subjects in an amount sufficient to suppress the migration of HIV (or other virally) infected T cells. An amount is said to be sufficient to "suppress" migration of T cells if the dosage, route of administration, etc. of the agent are sufficient to attenuate or prevent such migration.

30 The administration of a chimeric antibody may be for either a "prophylactic" or "therapeutic" purpose. When provided prophylactically, the

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chimeric antibody is provided in advance of any symptom of viral infection (for example, prior to, at, or shortly after) the time of such infection, but in advance of any symptoms of such infection). The prophylactic administration of the chimeric antibody serves to prevent or attenuate any subsequent migration of virally infected T cells. When provided therapeutically, the chimeric antibody is provided at (or shortly after) the detection of virally infected T cells. The therapeutic administration of the antibody serves to attenuate any additional migration of such T cells.

The antibodies of the present invention may, thus, be provided either prior to the onset of viral infection (so as to suppress the anticipated migration of infected T cells) or after the actual detection of such virally infected cells.

#### 6. Treatment of Asthma

In the thirteenth embodiment of the present invention a chimeric antibody capable of binding to ICAM-1 is used in the treatment of asthma.

The therapeutic effects of the anti-asthma agents of the present invention may be obtained by providing such agents to a patient by any suitable means (i.e. intravenously, intramuscularly, subcutaneously, enterally, or parenterally). It is preferred to administer the agents of the present invention intranasally as by nasal spray, swab, etc. It is especially preferred to administer such agents by oral inhalation, or via an oral spray or oral aerosol. When administering agents by injection, the administration may be by continuous infusion, or by single or multiple boluses.

The anti-asthma agents of the present invention are intended to be provided to recipient subjects in an amount sufficient to lessen or attenuate the severity, extent or duration of the asthma symptoms.

The chimeric antibodies of the present invention may be administered either alone or in combination with one or more additional anti-asthma agents (such as methylxanthines (such as theophylline), beta-adrenergic agonists (such

as catecholamines, resorcinols, saligenins, and ephedrine), glucocorticoids (such as hydrocortisone), chromones (such as cromolyn sodium) and anticholinergics (such as atropine), in order to decrease the amount of such agents needed to treat the asthma symptoms.

5           The administration of the chimeric antibodies of the present invention may be for either a "prophylactic" or "therapeutic" purpose. When provided prophylactically, the chimeric antibodies are provided in advance of any asthma symptom. The prophylactic administration of the chimeric antibody serves to prevent or attenuate any subsequent asthmatic response. When  
10       provided therapeutically, the chimeric antibody is provided at (or shortly after) the onset of a symptom of asthma. The therapeutic administration of the antibody serves to attenuate any actual asthmatic episode. The antibodies of the present invention may, thus, be provided either prior to the onset of an anticipated asthmatic episode (so as to attenuate the anticipated severity,  
15       duration or extent of the episode) or after the initiation of the episode.

C.   Administration of the Compositions of the Present Invention

          The therapeutic effects of chimeric antibodies capable of binding ICAM-1 may be obtained by providing to a patient an effective amount of a chimeric antibody which is substantially free of natural contaminants. The  
20       chimeric antibodies of the present invention disclosed herein are said to be "substantially free of natural contaminants" if preparations which contain them are substantially free of materials with which these products are normally and naturally found.

          The present invention extends to chimeric antibodies which may be  
25       produced either by an animal, or by tissue culture, or recombinant DNA means.

          In providing a patient with a chimeric antibody, the dosage of administered agent will vary depending upon such factors as the patient's age, weight, height, sex, general medical condition, previous medical history, etc.

In general, it is desirable to provide the recipient with a dosage of antibody which is in the range of from about 1 pg/kg to 10 mg/kg (body weight of patient), although a lower or higher dosage may be administered.

5 A chimeric antibody capable of binding to ICAM-1 may be administered to patients intravenously, intramuscularly, subcutaneously, enterally, topically inhaled, intranasally, or parenterally. When administering an antibody, the administration may be by continuous administration, or by single or multiple boluses.

10 A composition is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient patient. Such an agent is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient.

15 The chimeric antibodies of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby these antibodies are combined in a mixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in Remington's Pharmaceutical Sciences (16th ed., Osol, A., Ed., Mack, Easton PA (1980)). In order to form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of a chimeric antibody together with a suitable amount of carrier vehicle.

25 Additional pharmaceutical methods may be employed to control the duration of action. Controlled release preparations may be achieved through the use of polymers to complex or absorb the chimeric antibody. The controlled delivery may be exercised by selecting appropriate macromolecules (for example polyesters, polyamino acids, polyvinyl, pyrrolidone, ethylenevinylacetate, methylcellulose, carboxymethylcellulose, or protamine, 30 sulfate) and the concentration of macromolecules as well as the methods of

incorporation in order to control release. Another possible method to control the duration of action by controlled release preparations is to incorporate the chimeric antibody into particles of a polymeric material such as polyesters, polyamino acids, hydrogels, poly(lactic acid) or ethylene vinylacetate copolymers. Alternatively, instead of incorporating the antibody into polymeric particles, it is possible to entrap these materials in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatine-microcapsules and poly(methylmethacrylate) microcapsules, respectively, or in colloidal drug delivery systems, for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences (1980).

## MATERIAL METHODS

### 1. Incoming Cells

Hybridoma cell line R6-5-D6 producing anti-ICAM-1 antibody was provided by Boehringer Ingelheim Pharmaceuticals Inc. (Lot No. R6-5-D6 - E9-B2 0-29-86) and was grown up in antibiotic free Dulbecco's Modified Eagles Medium (DMEM) supplemented with glutamine and 5% foetal calf serum, and divided to provide both an overgrown supernatant for evaluation and cells for extraction of RNA. The overgrown supernatant was shown to contain murine IgG2a/kappa antibody. Cell culture supernatant was examined and confirmed to contain the antibody R6-5-D6.

### 2. Molecular Biology Procedures

Basic molecular biology procedures were as Maniatis *et al.* (1982) (Maniatis *et al.*, *Molecular Cloning*, Cold Spring Harbor, New York (1982)) with, in some cases, minor modifications. DNA sequencing was performed as described in Sanger *et al.* (1977) (Sanger *et al.*, *Proc. Natl. Acad. Sci.*



USA 74:5463-5467 (1977)) and the Amersham International Plc sequencing handbook. COS cell expression and metabolic labelling studies were as described in Whittle *et al.* (1987) (Whittle *et al.*, *Prot. Eng.* 1, 6:499-505 (1987)). Chinese Hamster Ovary (CHO) transfections and cell culture were performed as described in Gorman (1988) (Gorman, C., *DNA Cloning* 2:143-190 ed.(1988)) and Bebbington and Hentschel (1988) (Bebbington *et al.*, *DNA Cloning* 3:163-188 ed. (1988)).

### 3. Research Assays

#### 3.1. Assay for Secreted Antibody Light Chain

Supernatants from CHO cell lines were assayed for secreted light chain, after transfection with light chain expression vectors, as the first step in the development of stable cell lines producing whole chimeric antibody. The procedure was as follows:

96 well microtitre plates were coated with F(ab')<sub>2</sub> goat anti-human kappa light chain. The plates were washed with water and samples added and incubated for one hour at room temperature. The plates were washed and F(ab')<sub>2</sub> goat anti-human F(ab')<sub>2</sub> Horse radish peroxidase (HRPO) conjugate was then added and incubated for a further hour. Enzyme substrate was then added to reveal the reaction.

#### 3.2. Assembly Assays

Assembly assays were performed on supernatants from transfected COS cells and from transfected CHO cells to determine the amount of intact IgG present.

##### 3.2.1 COS and CHO Cells transfected with mouse genes

The assembly assay for intact mouse IgG in cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with F(ab')<sub>2</sub> goat anti-mouse IgG Fc. The plates were washed in water and samples added and incubated for 1 hour

at room temperature. The plates were washed and F(ab')<sub>2</sub> goat anti-mouse IgG F(ab')<sub>2</sub> (HRPO conjugated ) was then added. Enzyme substrate was then added to reveal the reaction. UPC10, a mouse IgG2a myeloma, was used as a standard.

5     **3.2.2 COS and CHO Cells transfected with Chimeric Genes**

The assembly assay for intact humanized anti-ICAM-1 in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with F(ab')<sub>2</sub> goat anti-human IgG Fc. The plates were washed and samples added and incubated for 1 hour at room temperature. The plates were washed and monoclonal mouse anti-human kappa chain was added and incubated for 1 hour at room temperature. The plates were washed and F(ab')<sub>2</sub> goat anti-mouse IgG Fc (HRPO conjugated) was added. Enzyme substrate was then added to reveal the reaction. Chimeric B72.3 (Bodmer *et al.*, Published International Patent Application WO 89/01783) (IgG4) and pooled, purified human IgG2 and IgG4 (Chemicon) were used initially as standards. Later, purified chimeric IgG4 anti-ICAM-1 was used as a standard for work with chimeric IgG1 anti-ICAM-1. The use of a monoclonal anti-kappa chain in this assay allows the amount of chimeric antibody to be read from the standards.

20     **3.3. Assay for Antigen Binding Activity**

**3.3.1 Direct Binding**

Material from COS and CHO cell supernatants and purified chimeric antibodies were assayed for anti-ICAM-1 antigen binding activity onto ICAM-1 positive cells in a direct assay. The procedure was as follows:

25     JY cells (a human B lymphoblastoid cell line which constitutively expresses ICAM-1 on the cell surface) were maintained in culture. Monolayers of JY cells were fixed onto 96 well ELISA plates using poly-L-lysine and paraformaldehyde, and the plates were blocked with a solution of bovine serum albumin in PBS. Samples were added to the monolayers and incubated

for 1 hour at room temperature. The plates were washed gently using PBS. F(ab')<sub>2</sub> goat anti-human IgG Fc (HRPO conjugated) or F(ab')<sub>2</sub> goat anti-mouse IgG Fc (HRPO conjugated) was then added as appropriate for humanized or mouse samples. Enzyme substrate was then added to reveal the reaction. The negative control for the cell-based assay was chimeric B72.3 (IgG4) or pooled, purified human IgG2 and IgG4 (Chemicon). The positive control was murine R6-5-D6 MAb.

### 3.3.2 Competition Binding

Monolayers of JY cells were prepared as in 3.3.1. Antibody samples were added and incubated overnight at 4°C. Biotinylated anti-ICAM-1 was added to all the wells. The mixture was left at room temperature for 2 hours. The plates were washed and either streptavidin-HRPO or streptavidin-betagalactosidase was added. After further incubation enzyme substrate was added to reveal the reaction.

### 3.3.3 Mixed Lymphocyte Reaction Assays

Peripheral blood was obtained from normal, healthy donors by venipuncture. The blood (7.5 ml) was layered over 7.5 ml of a Ficoll/Hypaque density gradient (Pharmacia, density = 1.078) room temperature and centrifuged at 1000 x g for 20 minutes. The cells were washed, counted on a hemacytometer, and suspended in RPMI-1640 culture medium (Gibco) containing 50 µg/ml gentamycin, 1 nM L-glutamine (Gibco) and 5% heat inactivated (55 C, 30 min) human AB sera (Flow Laboratories) (hereafter referred to as RPMI-culture medium).

Peripheral blood mononuclear cells (responder cells) were cultured in medium at  $6.25 \times 10^5$  cells/ml in Linbro round-bottomed microtiter plates (#76-013-05). Stimulator cells from a separate donor were irradiated at 1000R and cultured with the responder cells at the same concentration. Responder cells were added to wells, followed by the monoclonal antibodies and the stimulator cells were added last. The total volume per culture was 0.2

ml. Controls included responder cells alone. The culture plates were incubated at 37°C in a 5% CO<sub>2</sub>-humidified air atmosphere for 5 days. The wells were pulsed with 0.5 uCi tritiated thymidine (<sup>3</sup>HT) (New England Nuclear) for the last 18 hours of culture.

- 5           The cells were harvested onto glass fiber filters using an automated multiple sample harvester (Skatron, Norway), rinsing with distilled water. The filters were oven dried and counted in Beckman Ready Safe liquid scintillation cocktail on a LKB Betaplate liquid scintillation counter.

### 3.4 In Vivo Assays

#### 10   3.4.1 Modified Schwartzmann Reaction

- 15           The local Schwartzmann reaction in rabbit skin can be produced by an i.d. injection of endotoxin followed by an i.v. challenge injection of zymosan 18-24 hrs. later. The hemorrhagic necrosis that develops in the previously injected skin sites is characterized by microthrombi, intravascular neutrophil aggregation, platelet and fibrin deposition, vascular permeability increases and massive extravasation of erythrocytes (RBC). We have modified this protocol for use in the Cynomolgous monkey. Separate, distinct skin sites were injected i.d. with endotoxin (3 µg/site) or normal saline and 18 hours later these same sites were injected with zymosan (300 µg/site). The resulting inflammatory response was quantitated at 6 hours post-zymosan by measuring the increase in vascular permeability in the endotoxin-injected sites compared to the saline-injected sites using <sup>125</sup>I-BSA. The inhibitory effect of R6.5 (anti-ICAM-1), the IgG1, IgG2, and IgG4, chimerics of R6.5 and R15.7 (LFA-1 beta) were compared to that of normal mouse IgG. All IgG preparations were administered i.v. at 3 mg/kg prior to the zymosan injections.
- 20
- 25

### Results

#### 4. CDNA Library Construction

##### 4.1 mRNA Preparation and cDNA Synthesis

Cells were grown as described in Section 1 and  $1.4 \times 10^9$  cells harvested and mRNA extracted using the guanidinium/LiCl extraction procedure. cDNA was prepared by priming from Oligo-dT to generate full length cDNA. The cDNA was methylated and EcoRI linkers added for cloning.

#### 4.2 Library Construction

The cDNA library was ligated to pSP64 vector DNA which had been EcoRI cut and the 5' phosphate groups removed by calf intestinal phosphatase (EcoRI/CIP). The ligation was used to transform high transformation efficiency *Escherichia coli* HB101 (*E. coli* HB101) from Bethesda Research Labs (BRL) in the case of the light chain and *E. coli* LM1035 prepared by electroporation (Dower *et al.*, *Nucl. Acids Res.* 16:6127 (1988)) in the case of the heavy chain. cDNA libraries were prepared. 11600 colonies were screened for the light chain and 25000 colonies were screened for the heavy chain.

#### 5. Screening

*E. coli* colonies positive for either heavy or light chain probes were identified either by oligonucleotide screening using the oligonucleotide: 5' TCCAGATGTAACTGCTCAC for the light chain, which is complementary to a sequence in the mouse kappa constant region, or by using a 980 bp BamHI-EcoRI restriction fragment of a previously isolated mouse IgG2a constant region clone. 6 light chain and 10 heavy chain clones were identified and taken for second round screening. Positive clones from the second round of screening were grown up and DNA prepared. The sizes of the gene inserts were estimated by gel electrophoresis and DNA inserts of a size capable of containing a full length cDNA were sequenced.

## 6. DNA Sequencing

DNA sequence for the 5' untranslated regions, signal sequences, variable regions and 3' untranslated regions of full length cDNAs were obtained and are given in Figure 1 for the light chain and Figure 2 for the heavy chain.

## 7. Construction of cDNA Expression Vectors

CellTech expression vectors are based on the plasmid pEE6-hCMV as shown in Figure 3 (Bebbington, C.R., Published International Patent Application WO 89/01036). A polylinker for the insertion of genes to be expressed has been introduced after the major immediate early promoter/enhance of the human Cytomegalovirus (hCMV). Marker genes for selection of the plasmid in transfected eukaryotic cells can be inserted as BamHI cassettes in the unique BamHI site of pEE6-hCMV. It is usual practice to insert the neo and gpt markers prior to insertion of the gene of interest, whereas the GS marker is inserted last because of the presence of internal EcoRI sites in the cassette. The selectable markers are expressed from the SV40 late promoter which also provides an origin of replication so that the vectors can be used for expression in the COS cell transient expression system. The mouse sequences were excised as EcoRI fragments and cloned into either EE6-hCMV-neo for the light chain (Figure 4) and into EE6-hCMV-gpt for the heavy chain (Figure 5).

## 8. Expression of cDNAs in COS cells

Plasmids pAL5 (Figure 4) and pAL6 (Figure 5) were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to JY cells (Figure 6). Metabolic labelling experiments using <sup>35</sup>S methionine showed expression and assembly of heavy and light chains.

## 9. Construction of Chimeric Genes

Construction of chimeric genes followed a previously described strategy (Whittle *et al.* 1987, (Whittle *et al.*, *Prot. Eng.* 1, 6:499-505 (1987)). A restriction site near the 3' end of the variable domain sequence is identified and used to attach an oligonucleotide adapter which codes for the remainder of the mouse variable region and includes a suitable restriction site for attachment to the constant region of choice.

### 9.1 Light Chain Gene Construction

The mouse light chain cDNA sequence showed an SfaNI site near the 3' end of the variable region. The majority of the sequence of the variable region was isolated as a 397 bp. EcoRI-SfaNI fragment. An oligonucleotide adapter was designed to replace the remainder of the 3' region of the variable region from the SfaNI site and to include the 5' residues of the human constant region up to and including a unique NarI site which had been previously engineered into the constant region. The linker was ligated to the human C<sub>K</sub> gene in NarI cut pRB32 and the SfaNI-EcoRI adapted C<sub>K</sub> fragment was purified from the ligation mixture. The constant region was ligated with the EcoRI-SfaNI cut variable region DNA into an EcoRI/CIP pEB6-hCMV-neo treated vector in a three way reaction. Clones were isolated after transformation into *E. coli* and the linker and junction sequences were confirmed by DNA sequencing.

Figure 7 shows the strategy for construction of the chimeric light chain.

### 9.2 Heavy Chain Gene Construction

#### 9.2.1. Choice of Heavy Chain Gene Isotype

Chimeric heavy chain genes coding for both human IgG2 and IgG4 isotypes were constructed.

### 9.2.2. Gene Construction

The heavy chain cDNA sequence showed a *Ban*I site near the 3' end of the variable region. The majority of the sequence of the variable region was isolated as a 424bp *Eco*RI/*CIP*/*Ban*I fragment. An oligonucleotide  
5 adapter was designated to replace the remainder of the 3' region of the variable region from the *Ban*I site up to and including a unique *Hind*III site which had been previously engineered into the first two amino acids of the constant region. The linker was ligated to the  $C_H$  gene fragments in *Hind*III cut pRB41 and pRB21 and the *Ban*I-*Bam*HI adapted constant region fragments  
10 were purified from the ligation mixture. The *Eco*RI-*Ban*I variable region fragment was ligated to each of the constant regions and into the expression vector (*Eco*RI/*Bcl*I/*CIP* treated pEE6-hCMV-gpt) via a three way ligation. Clones were isolated after transformation into *E. coli* HB101 and the linker and junction sequences were confirmed by DNA sequencing. Figure 8 shows  
15 the strategy for the construction of the chimeric IgG2 and IgG4 heavy chains and Figure 9 shows an outline plasmid map of pAL8 (IgG2) and pAL9 (IgG4).

### 9.2.3 IgG1 Heavy Chain Gene Construction

Plasmid pE1001 is an expression vector based on pEE6.hCMV gpt.  
20 It contains the human IgG1 constant region gene. The *Apa*I site which occurs at the 5th and 6th codon of the CH1 domain is unique in this vector, as is a *Hind*III site 3' to the hCMV promoter. The VH region of the anti-ICAM-1 heavy chain gene along with the sequence encoding the first five residues of human CH1 was isolated from pAL9 and inserted into pE1001, previously cut  
25 with *Hind*III and *Apa*I, to give pJA200. The CH1 residues carried over from pAL9 are identical to those for IgG1 therefore no novel sequence is generated at the V-C junction.



## **10. Construction of Chimeric Expression Vectors**

### **10.1 GS Separate Vectors**

GS versions of pAL7, pAL8 and pAL9 (Figures 7, 8 and 9) were constructed by replacing the neo and gpt BamHI cassettes with a 5.9Kbp cassette containing the GS gene capable of being expressed from the SV40 late promoter (See Figures 7, 10-12 for plasmid drawings).

## **11. Expression of Chimeric Genes**

### **11.1 Expression in COS Cells**

The chimeric antibody plasmid pAL7 (cL), with either pAL8 (cHIgG2), pAL9 (cHIgG4), or pJA200 (CHiG1) was co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to the JY human B-cell line. Metabolic antibody which bound to the JY human B-cell line. Metabolic labelling experiments using <sup>35</sup>S methionine showed expression and assembly of heavy and light chains (Figure 13). All chimeric antibodies bound well to the JY cells. However, the competition assay showed that antibody derived from the cL/cHIgG2 or the cL/cHIgG4 combination did not compete as well as the biotinylated mouse antibody for ICAM-1. The cL/cHIgG1 antibody competed better than the cL/cHIgG2 or cL/cHBIgG4 antibodies.

### **11.2 Expression in Chinese Hamster Ovary (CHO) Cells**

Stable cell lines were prepared as follows: Chimeric light chain expression vectors pAL7 and pAL10 containing either the neo or GS markers were transfected into CHO-K1 cells by the CaPO<sub>4</sub> precipitation procedure. After growth on selective medium, positive cell lines were identified and their specific production rates measured using and ELISA format assay for detection of secreted light chain. The two cell lines secreting the highest levels of light chain (see table below) were selected and retransfected with either pAL8 or pAL9 to introduce the IgG2 and IgG4 chimeric heavy chain genes along with the gpt marker.

5

CELL LINE	SELECTABLE MARKER	SPECIFIC PRODUCTION RATE (pg./cell/day)
24	neo	2.1
46	neo	0.9
25	GS	2.0
27	GS	2.3

10

Around 24 lines shown to be secreting chimeric antibody by assembly assay ELISA were taken from each transfection. Specific production rates were measured and the 8 lines with the highest specific production rates are shown below.

15

20

CELL LINE	SPECIFIC PRODUCTION RATE (pg./Cell/day)
neo 24 G2-9	6.9
neo 24 G2-233	9.0
neo 24 G4-19	3.0
neo 24 G4-22	4.2
neo 24 G4-24	7.5
GS 25 G2-12	11.7
GS 25 G4-5	2.1
GS 27 G4-8	2.3

The chimeric IgG1 heavy chain expression vector pJA200 containing the gpt marker was transfected into the chimeric light chain expressing cell line neo24 by the CaPO4 precipitation method. Specific production rates were measured, the 4 best lines are shown below.

CELL LINE	SPECIFIC PRODUCTION RATE (pg./cell/day)
24.1.48	3.75
24.2.7	1.2
24.1.41	1.3
24.2.11	1.75

## 12. Purification of Chimeric Antibody

Antibody was purified from 2x1L harvests of supernatant from roller cultures for cell lines GS 25 G2-12, neo 24 G4-24 and 24.1.48, and also from 2x0.5L harvests from neo 24 G2-9 by affinity chromatography using Protein A Sepharose. Cell culture supernatants were adjusted to pH8.8 with 0.2M sodium glycinate and applied to a protein A Sepharose column which had been pre-equilibrated with glycine/glycinate buffer at pH8.8. After the samples had been loaded the column was washed with equilibration buffer. The antibody was then eluted by applying a solution with a decreasing pH gradient consisting of 0.2M disodium hydrogen phosphate and 0.1M citric acid. Antibody containing fractions were pooled and the pH adjusted to 6.5 the samples were then dialyzed against phosphate buffered saline (PBS). Purity and correct assembly of the antibody was tested by reducing and non-reducing SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 14) and by high performance liquid chromatography (HPLC) gel filtration (Figure 15). Identity was confirmed by N-terminal amino acid sequencing and amino acid composition analysis.

## 13. Analysis of Purified Antibody

### 13.1 Results of Direct Binding and Competitive Binding Assays

All chimeric antibodies bound well to the JY cells (Figures 16 and 17). However, in competition assays only the cL/CHlgG1 antibody competed nearly as well as the mouse antibody against the biotinylated mouse antibody for binding to ICAM (Fig. 18). The cL/CHlgG4 antibody showed about 30% of

the inhibitory activity of the mouse antibody (Fig. 19) and the cL/CHlgG2 antibody showed about 10% of the inhibitory activity of the mouse antibody (Fig. 20).

5 These data show chimeric anti-ICAM-1 antibodies derived from R6-5-D6 have different antigen binding activities depending on isotype. The IgG1 antibody is nearly as avid as the mouse parent antibody. The IgG4 antibody as 30% of the competitive binding activity of the mouse antibody, the IgG2 has 10% relative activity. This result is unexpected as all these antibodies have identical binding sites. We attribute these differences to avidity  
10 alterations imposed on the antibodies by the differing hinge flexibility of the isotypes. Affinity measurements of the chimeric IgG4 Fab have shown that this has the same affinity as the mouse Fab thus confirming that it is the avidity that has altered in construction of the chimeric IgG4.

### 13.2 Results of Mixed Lymphocyte Reaction Assays

15 The MRL which is an in vitro model of transplantation was inhibited by the chimeric IgG4 and IgG1 to comparable extent as the mouse r6-5-6D antibody. This shows that the chimeric MAb will inhibit specific immunological events and can thus be used in in vivo autoimmune and transplantation settings (see Fig. 21).

### 20 13.3 Results of Modified Schwartzmann Reaction Assays

The primate Schwartzmann reaction was set up to test granulocyte function in vivo in the presence of an anti-ICAM-1 MAb. The chimeric IgG1 was slightly more active than the mouse MAb which in turn was slightly more active in inhibiting activated neutrophil mediated vascular leakage than the  
25 chimeric IgG4 in the primate model. This demonstrates that the chimeric anti-ICAM-1's will be effective in mitigating neutrophil mediated damage associated with reperfusion injury and other acute inflammatory disorders (see Fig. 22).

**CLAIMS:**

1. A chimeric antibody molecule comprising heavy and/or light chain variable regions of an anti-ICAM-1 antibody.

5 2. The chimeric antibody molecule of claim 1 which is a humanized chimeric antibody.

3. The chimeric antibody molecule of claims 1 or 2 having attached to it an effector or reporter molecule.

4. The chimeric antibody molecule of claims 1-3 comprising at least one chimeric heavy chain and at least one chimeric light chain.

10 5. The chimeric antibody of claims 2-4 comprising IgG human constant region domains.

6. The chimeric antibody of claim 5 comprising IgG2 or IgG4 human constant region domains.

15 7. The chimeric antibody of claim 5 comprising IgG1 human constant region domains.

8. The chimeric antibody of claims 1-7 wherein said chimeric antibody has binding specificity for the same or similar epitope(s) as the R6-5-D6 antibody.

20 9. The chimeric antibody of claims 1-8 wherein said chimeric antibody is derived from the R6-5-D6 antibody.

10. A DNA sequence encoding the heavy or light chain variable region of an anti-ICAM-1 antibody.
11. A DNA molecule encoding a chimeric heavy or light chain comprising the variable region of an anti-ICAM-1 antibody.
- 5 12. The DNA of claim 11 which codes for a humanized chimeric heavy or light chain.
13. The DNA of claim 12 coding for a humanized chimeric heavy chain comprising human IgG constant region domains.
- 10 14. The DNA of claim 13 comprising a human IgG2, preferably IgG4 or especially IgG1 constant region domains.
- 15 15. A vector comprising DNA according to any of claims 10-14.
16. An expression vector comprising in operative combination DNA coding for a chimeric anti-ICAM-1 light chain and a chimeric anti-ICAM-1 heavy chain.
- 15 17. A host cell transformed with a vector according to claims 15 or 16.
18. A process for the production of an anti-ICAM-1 humanized chimeric antibody comprising:
- 20 (1) producing an expression vector comprising an operon having a DNA sequence which encodes an antibody heavy or light chain wherein at least one of the CDRs of the variable domain are derived from a non-human (rodent) anti-ICAM-1 antibody and the remaining immunoglobulin-

- 53 -

derived parts of the antibody chain are derived from a human immunoglobulin;

- 5 (2) producing an expression vector comprising an operon having a DNA sequence which encodes a complementary antibody light or heavy chain wherein at least one of the CDRs of the variable domain are derived from a rodent (non-human) anti-ICAM-1 antibody and the remaining immunoglobulin-derived parts of the antibody chain are derived from a human immunoglobulin;
- 10 (3) transfecting a host cell with each vector; and  
(4) culturing the transfected cell line to produce the chimeric antibody.

19. A method of treatment comprising administering an effective amount of an antibody product according to any one of claims 1-9 to a human or animal subject.

- 15 20. A method for treating inflammation resulting from a response of the specific defense system in a mammalian subject which comprises providing to a subject in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress said inflammation, wherein said anti-inflammatory agent is a chimeric antibody capable of binding ICAM-1.

- 20 21. The method of claim 20, wherein said chimeric antibody is one or more of the antibodies of claims 1-9.

22. The method of claim 20, wherein said inflammation is a delayed type hypersensitivity reaction.

- 25 23. The method of claim 20, wherein said inflammation is a symptom of psoriasis.

24. The method of claim 20, wherein said inflammation is a symptom of an autoimmune disease.

5 25. The method of claim 24, wherein said autoimmune disease is selected from the group consisting of Reynaud's syndrome, autoimmune thyroiditis, EAE, multiple sclerosis, rheumatoid arthritis and lupus erythematosus.

26. The method of claim 20, wherein said inflammation is in response to organ transplant rejection.

10 27. The method of claim 26, wherein said organ transplant is a kidney transplant.

28. The method of claim 20, wherein said inflammation is in response to tissue graft rejection.

15 29. The method of claims 20 which additionally comprises the administration of an agent selected from the group consisting of: an antibody capable of binding to LFA-1; a functional derivative of said antibody, said functional derivative being capable of binding to LFA-1; and a non-immunoglobulin antagonist of LFA-1.

20 30. A method for treating inflammation resulting from a response of the non-specific defense system in a mammalian subject which comprises providing to a subject in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress said inflammation, wherein said anti-inflammatory agent is a chimeric antibody capable of binding ICAM-1.

31. The method of claim 30 wherein said inflammation is associated with a condition selected from the group consisting of: adult respiratory



distress syndrome; multiple organ injury syndrome secondary to septicemia; multiple organ injury syndrome secondary to trauma; reperfusion injury of tissue; acute glomerulonephritis; reactive arthritis; dermatosis with acute inflammatory components; a central nervous system inflammatory disorder  
5 e.g. stroke; thermal injury; hemodialysis; leukapheresis; ulcerative colitis; Crohn's disease; necrotizing enterocolitis; granulocyte transfusion associated syndrome; and cytokine-induced toxicity.

32. The method of claims 30 or 31 wherein said chimeric antibody is one or more of the antibodies of claims 1-9.

10 33. A method of suppressing the metastasis of a hematopoietic tumor cell, said cell requiring a functional member of the LFA-1 family for migration, which method comprises providing to a patient in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress said metastasis, wherein said anti-inflammatory agent is a chimeric antibody  
15 capable of binding ICAM-1.

34. The method of claim 33, wherein said chimeric antibody capable of binding to ICAM-1 is selected from the group of antibodies of claims 1-9.

20 35. A method of suppressing the growth of an ICAM-1-expressing tumor cell which comprises providing to a patient in need of such treatment an amount of a toxin sufficient to suppress said growth, said toxin consists of a toxin-derivatized chimeric antibody capable of binding to ICAM-1.

36. A method for treating viral infection in an individual in need of such treatment, wherein said method comprises providing to said individual

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an amount of a chimeric antibody capable of binding ICAM-1 sufficient to suppress viral infection.

5           37.   The method of claim 36, wherein said virus is a rhinovirus of the major serotype within the genus Picornaviridae, a group A coxsackievirus, or a Mengo virus.

          38.   The method of claim 37 wherein said virus is a rhinovirus of the major serotype.

          39.   The method of any of claims 36-38 wherein said chimeric antibody is at least one of the antibodies of claims 1-9.

10           40.   A method for suppressing the infection of leukocytes with HIV, which comprises administering to a patient exposed to or infected by HIV, an effective amount of an HIV-1 infection suppression agent, said agent being a chimeric antibody capable of binding to ICAM-1.

          41.   The method of claim 40 wherein said HIV is HIV-1.

15           42.   The method of any one of claims 40 or 41, wherein said chimeric antibody is at least one of the antibodies of claims 1-9.

20           43.   A method for suppressing the extravascular migration of a virally infected leukocyte in a patient having such a leukocyte, which comprises administering to said patient an effective amount of an anti-migration agent, said agent being a chimeric antibody capable of impairing the ability of said leukocyte to bind to ICAM-1.

          44.   The method of claim 43, wherein said virally infected cells are infected with HIV.

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45. The method of claims 43 or 44, wherein said chimeric antibody is at least one of the antibodies of claims 1-9.

46. A method for treating asthma in an individual in need of such treatment, wherein said method comprises providing to said individual an amount of a chimeric antibody capable of binding ICAM-1 sufficient to suppress asthma.

47. The method of claim 46 wherein said chimeric antibody is at least one of the antibodies of claims 1-9.

48. The method of any one of claims 19-47 wherein said humanized chimeric antibody is administered by enteral means, parenteral means, topical means, inhalation means or intranasal means.

49. The method of claim 48 wherein said humanized chimeric antibody is administered prophylactically.

50. The method of claim 48 wherein said humanized chimeric antibody is administered therapeutically.

51. The method of claims 48, 49 or 50 wherein said parenteral means is intramuscular, intravenous or subcutaneous.

52. A pharmaceutical composition comprising the anti-inflammatory agent of any one of claims 1-9 in combination with a pharmaceutically acceptable carrier.

53. The pharmaceutical composition of claim 52 in combination with at least one other immunosuppressive agent.

54. A method of diagnosing an ICAM-1-expressing tumor cell in a mammalian subject which comprises:

(a) administering to said subject a composition containing a detectably labeled chimeric antibody capable of binding to ICAM-1, and

5 (b) detecting said chimeric antibody bound to said ICAM-1.

55. A method of diagnosing inflammation in a mammalian subject which comprises:

(a) incubating a sample of tissue of said subject with a composition containing a detectably labeled chimeric antibody capable of binding to a cell which expresses ICAM-1, and

10 (b) detecting said chimeric antibody bound to said cell.

$\frac{1}{24}$ [illegible]

Fig. 1(i)

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```

345      TTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGAGTTATTTCTGCTCTCAA
      -----+-----+-----+-----+-----+-----+-----+-----+
      AAGTGTGAGTTCTAGTCGTCCTCACCTCCGACTCCTAGACCCCTCAAATAAAGACGAGAGTT
      F T L K I S R V E A E D L G V Y F C S O
      C
      404

      AGTACACATGTTCCCTCTCAAGTTCGGAGGGGGGACCAAGCTGGAAATAAAACGGGCTGAT
      -----+-----+-----+-----+-----+-----+-----+-----+
      TCATGTGTACAAGGAGAGTGCAAGCCTCCCCCTGGTTCGACCTTTATTTTGCCCGACTA
      S T H V P L T F G G G T K L E I K R A D
      C
      464

      GCTGCACCAACTGTATCCATCTTCCACCATCCAGTGAGCAGTTAAACATCTGGAGGXGCC
      -----+-----+-----+-----+-----+-----+-----+-----+
      CGACGTGGTTGACATAGGTAGAAGGGTGGTAGGTCACCTCGTCAATTGTAGACCTCCXCGG
      A A P T V S I F P P S S E O L T S G G A
      C
      524

      TCAGTCGTGTGCTTCTTGAACAACCTTACCCCAAGACATCAATGTCAGTGGAG
      -----+-----+-----+-----+-----+-----+-----+-----+
      AGTCAGCACACGAAGAACTTGTGTAAGATGGGGTTCTGTAGTTACAGTTCACTTC
      S V V C F L N N F Y P K D I N V K W K
      C
      581

```

## KEY:

The protein sequence overlined is the leader sequence.

The protein sequences underlined are the CDR's.

The protein sequence underlined and overlined is part of the constant domain

The starred (\*) sequence was confirmed from protein sequencing.

Fig. 1(ii)

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## DNA Sequence of Anti-Icam Heavy chain with translation

[illegible]

Fig. 2(i)

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```

301      ACTGTTGACAAATCCTCCAACACAGCCTATTGGAACCTGCCAGATTGACATCTGAGGAT
a      T V D K S S N T A Y L E L A R L T S E D
361      TCTGCCATCTATTACTGTGCAAGAGGGGGATGGTTACTACTCTCCTTTGACTACTGGGGC
a      A G C G G T A G A T A T G A C A C G T T C T C C C C C T A C C A A T G A T G A G A G G A A A C T G A T G A C C C C G
421      C A A G G C A C C A C T C T C A G T C T C T C A G C C A A A A C A A C A G C C C C A T C G G T C T A T C C A C T G
a      Q G T T L T V S S A K T T A P S V Y P L
481      G C C C C T G T G T G G A G A T A C A A C T G G C T C C T C G G T G A C T C T A G G
a      A P V C G D T T G S S S V T L ?

```

KEY:

The protein sequence in overlined is the leader sequenceThe protein sequences underlined are the CDR'sThe protein sequence underlined and overlined is part of the constant domain

Fig. 2(ii)

SUBSTITUTE SHEET



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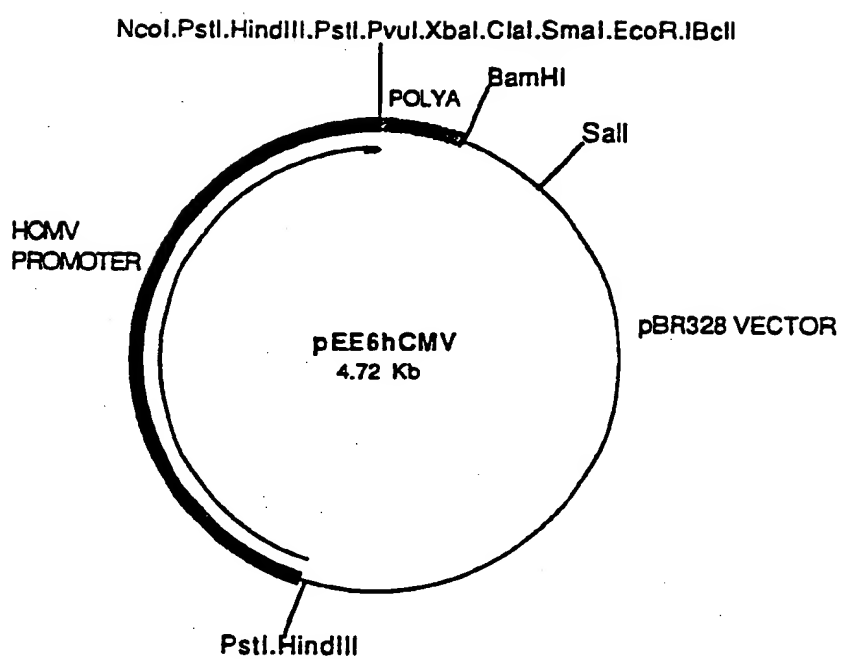


Fig. 3

SUBSTITUTE SHEET

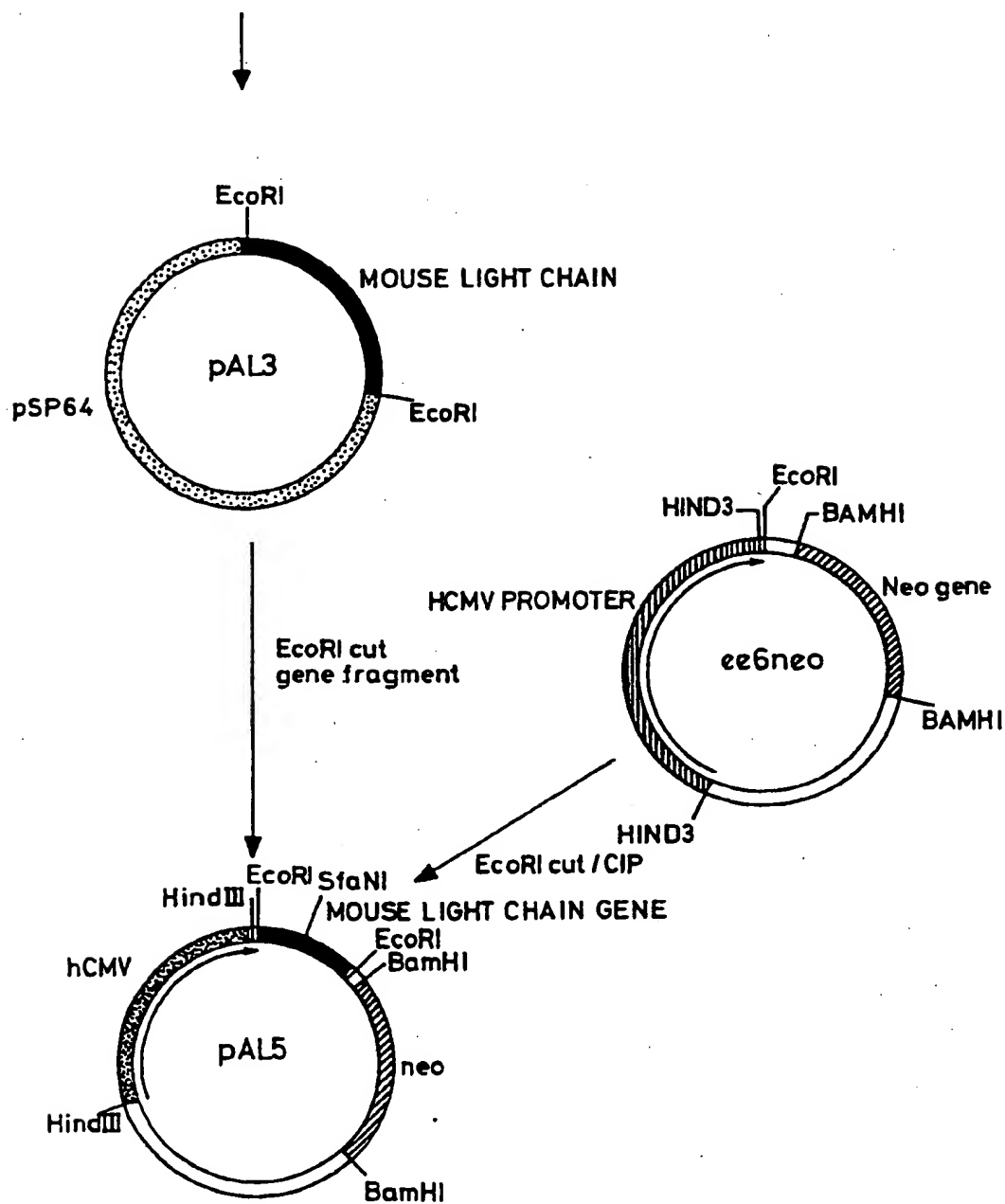
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ADD EcoRI LINKERS

CLONE

SCREEN

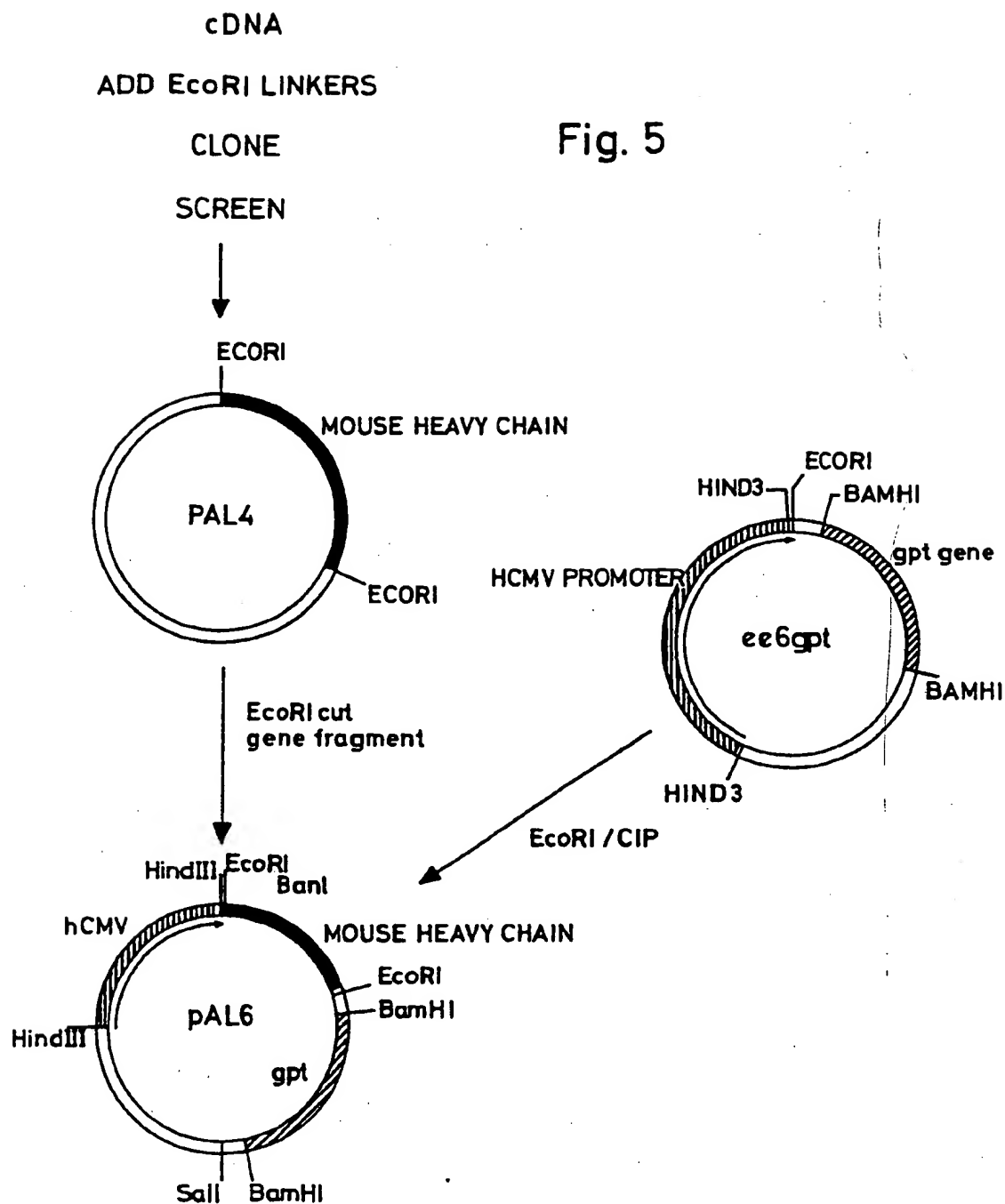
Fig. 4



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Fig. 5



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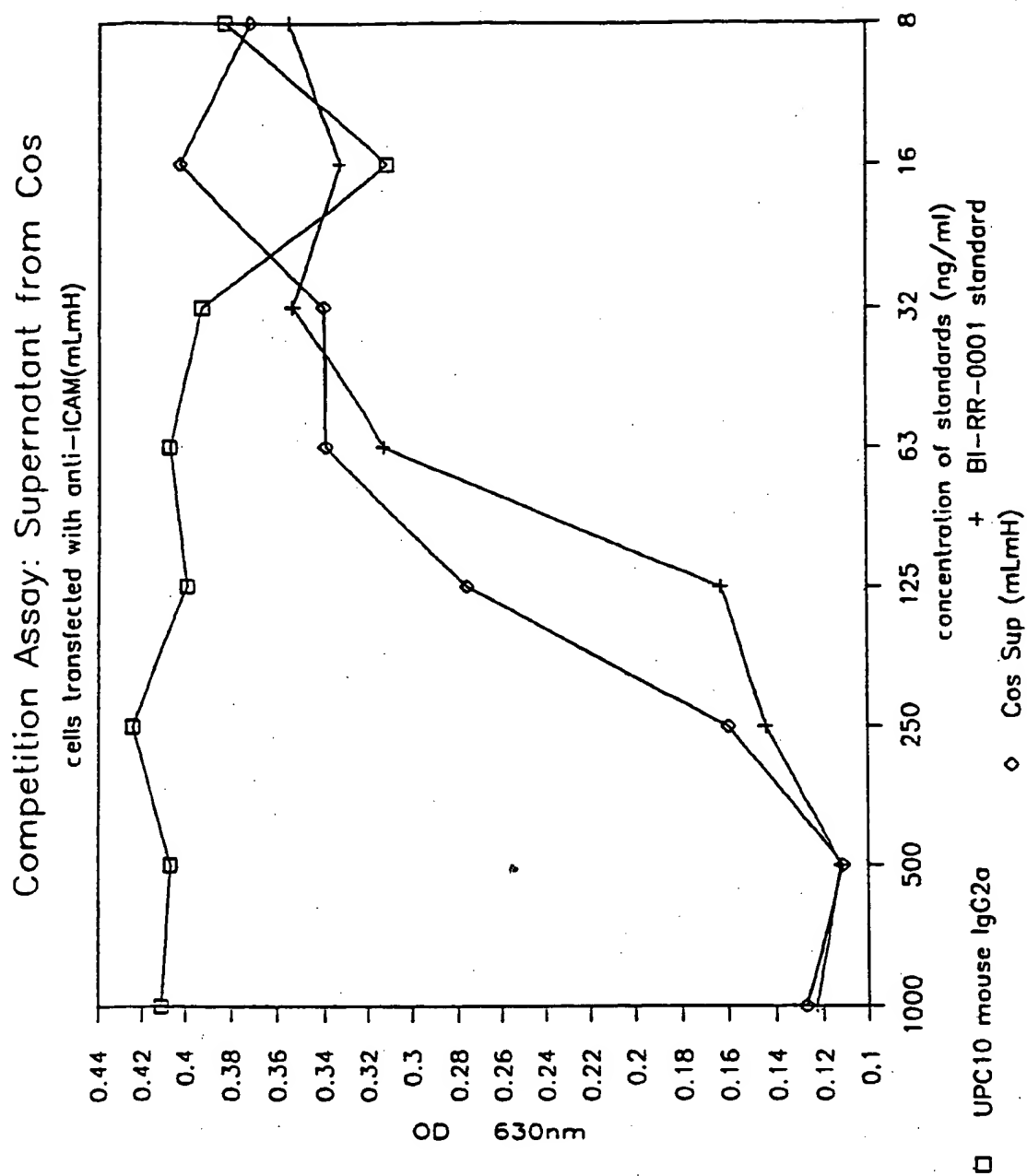


Fig. 6

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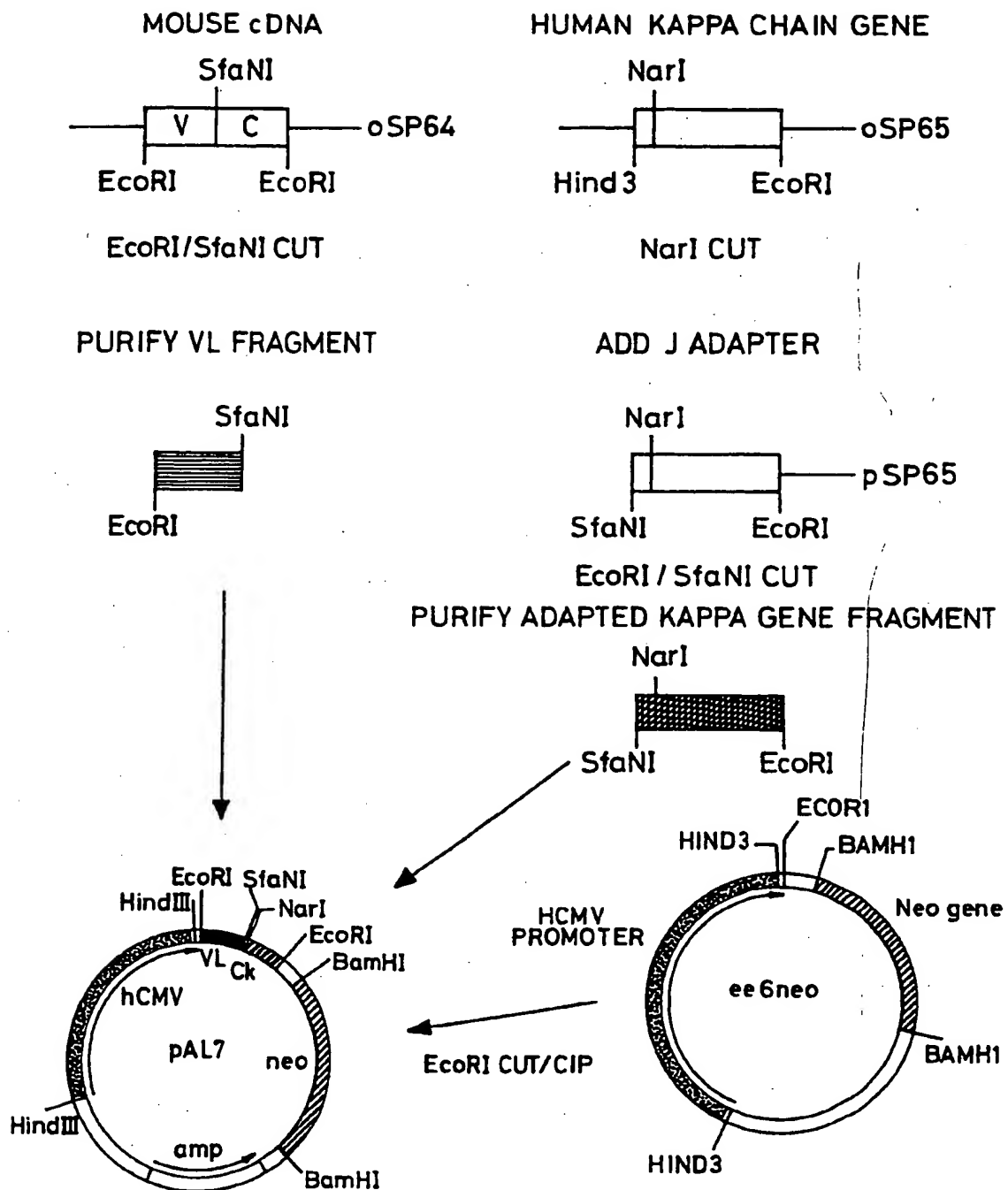


Fig. 7

SUBSTITUTE SHEET

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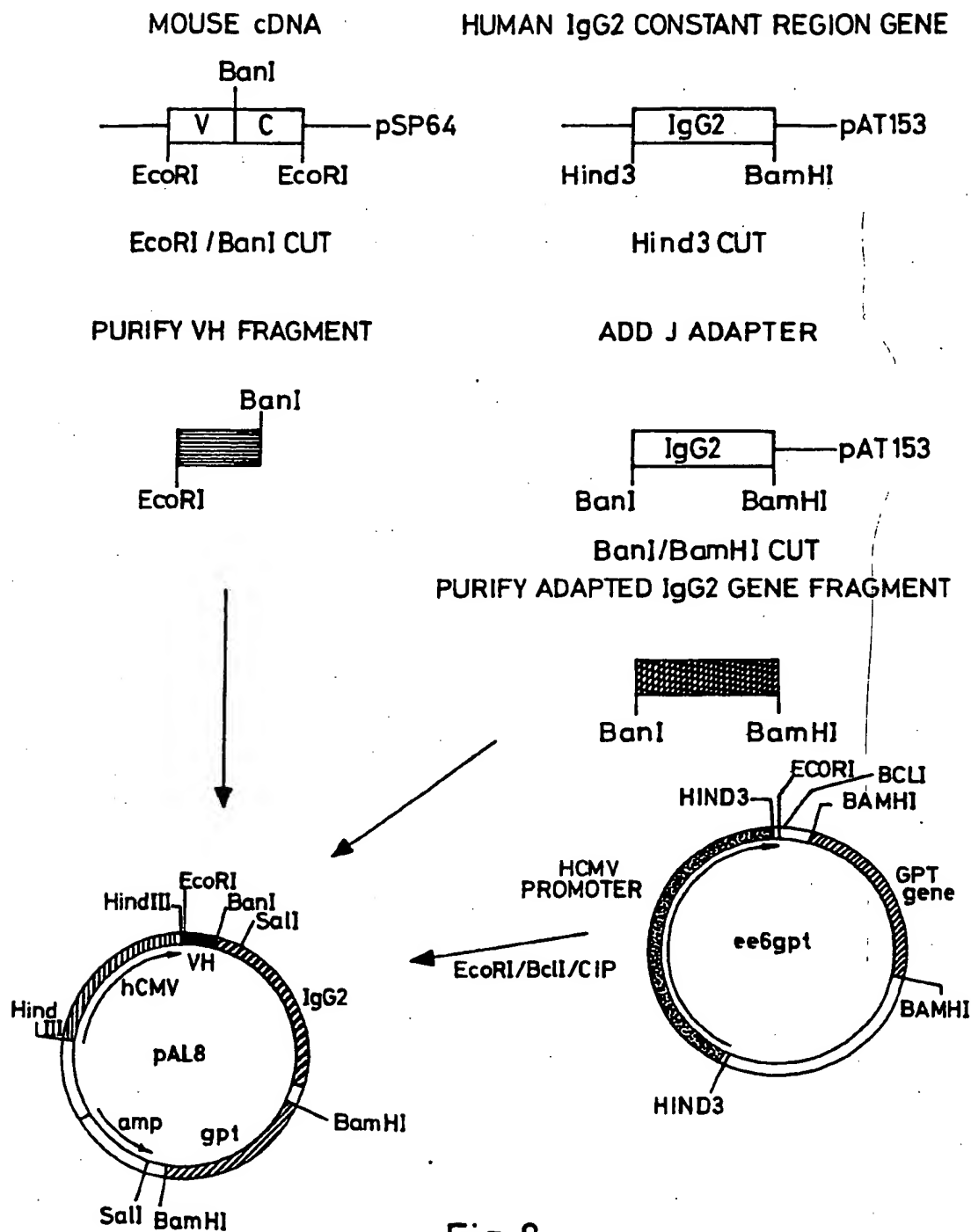
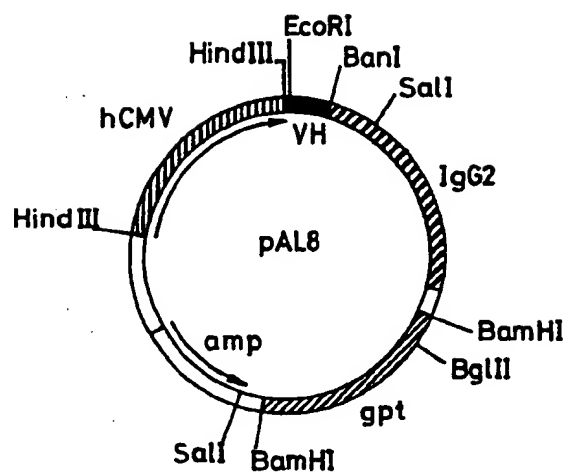


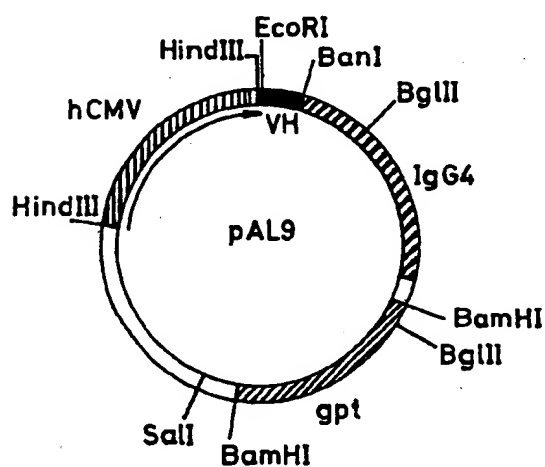
Fig. 8

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pAL8, CHIMAERIC HEAVY CHAIN EXPRESSION VECTOR  
IgG2 isotype



pAL9, CHIMAERIC HEAVY CHAIN EXPRESSION VECTOR  
IgG4 isotype

Fig. 9

SUBSTITUTE SHEET

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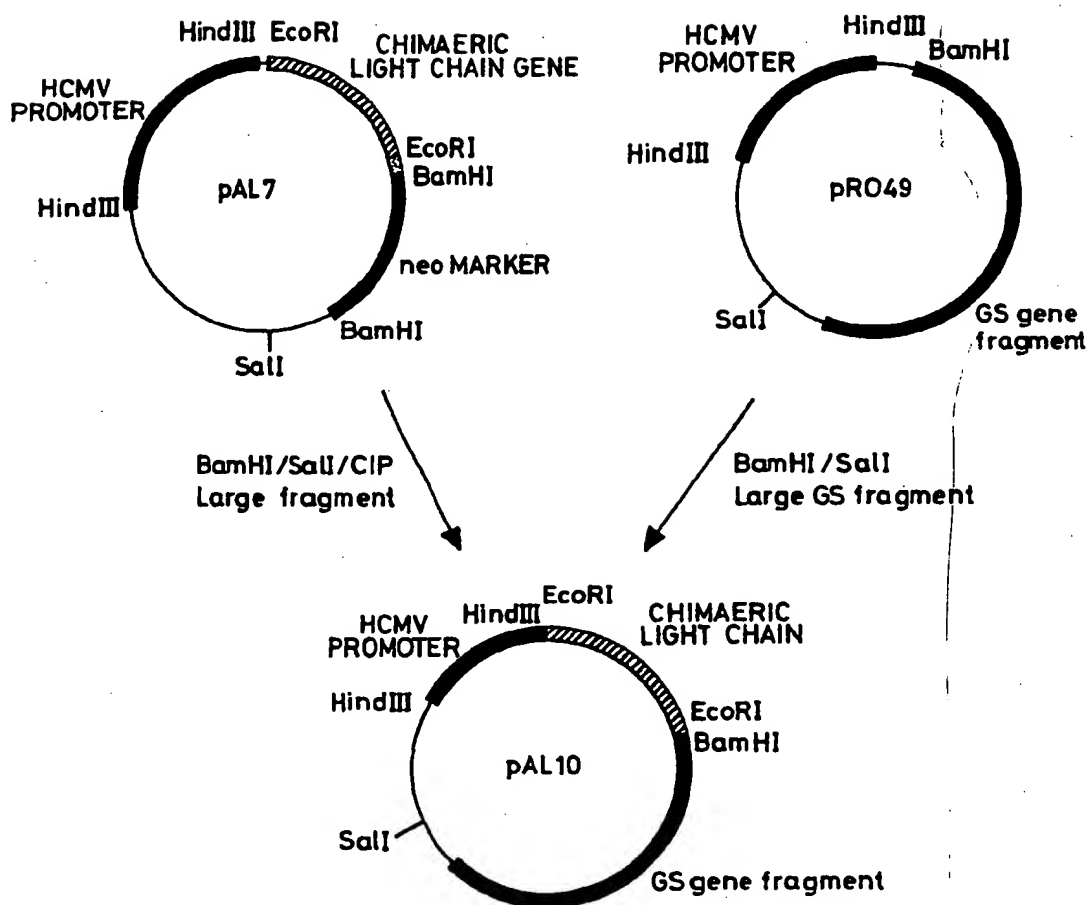


Fig. 10

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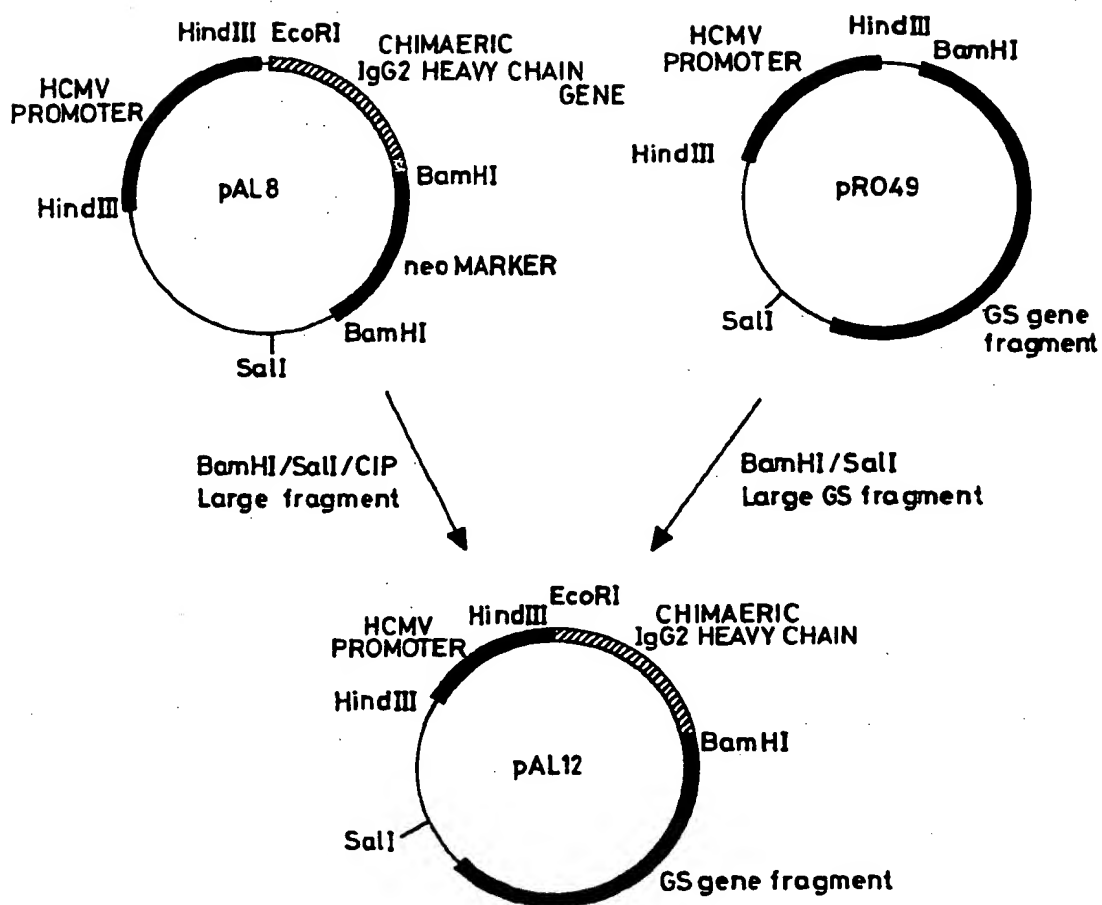


Fig. 11

SUBSTITUTE SHEET

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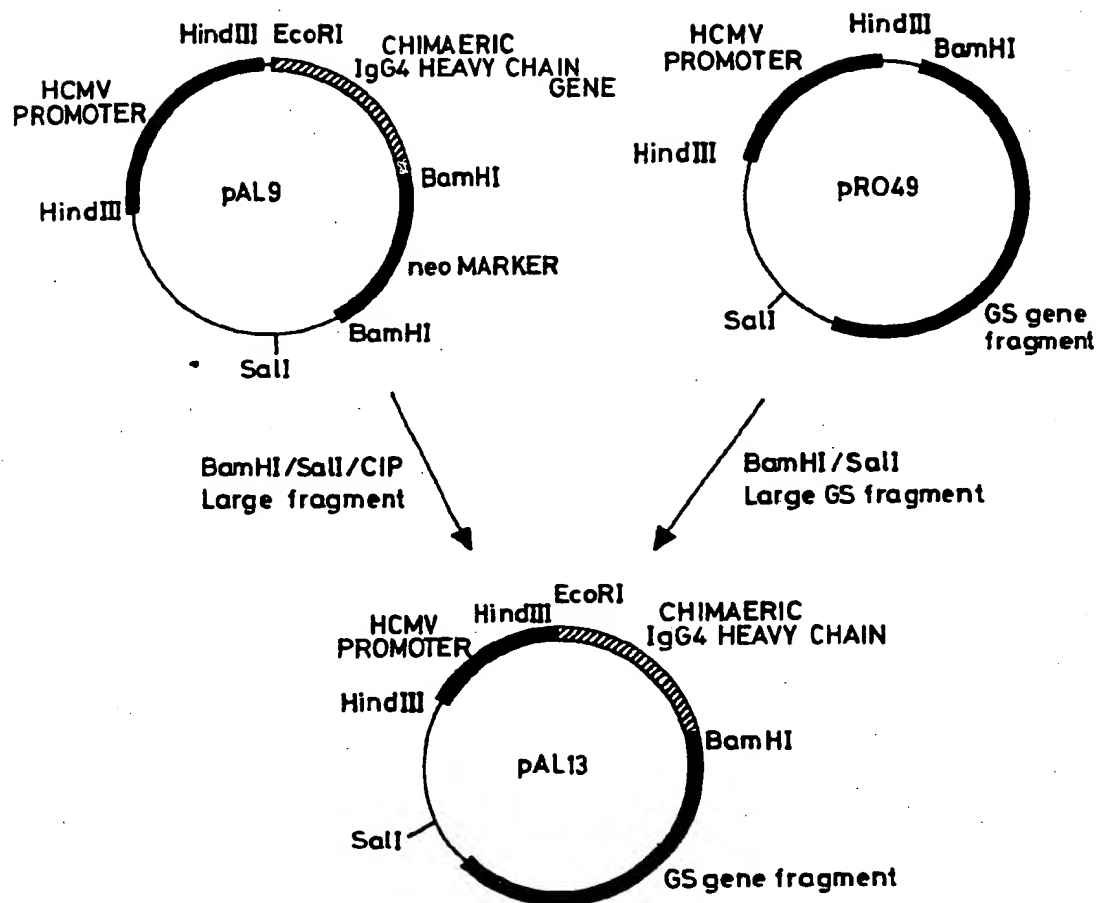


Fig. 12

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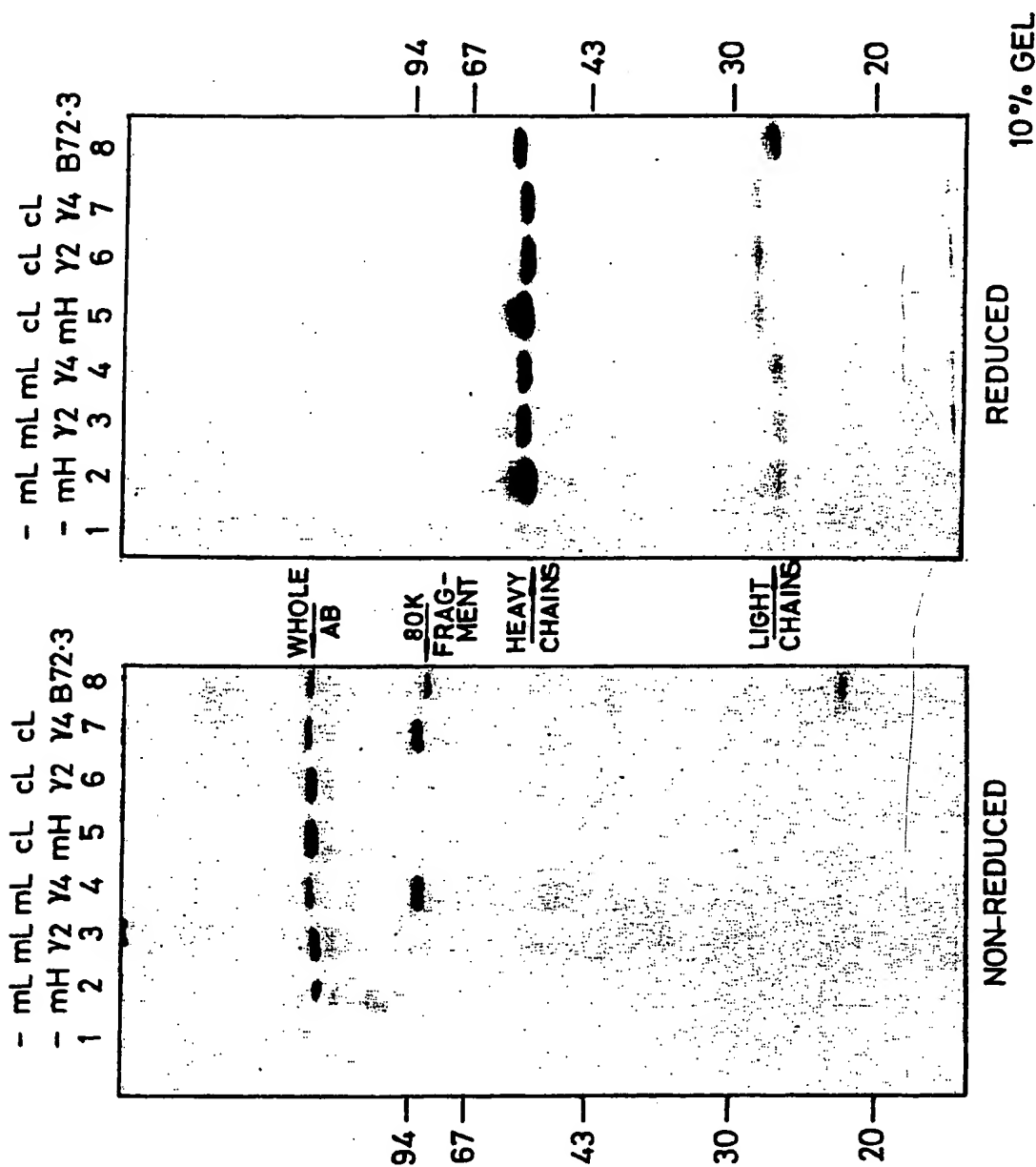


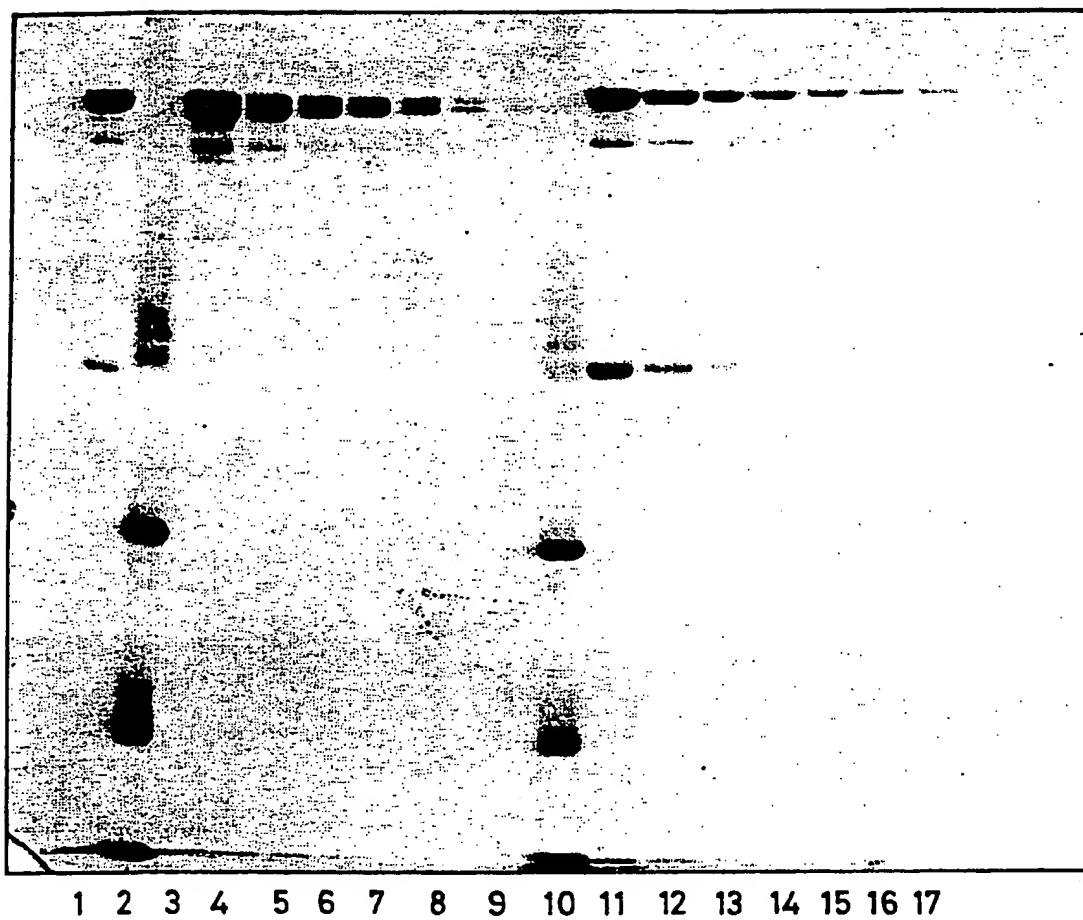
Fig. 13

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Fig. 14 (i)

A.

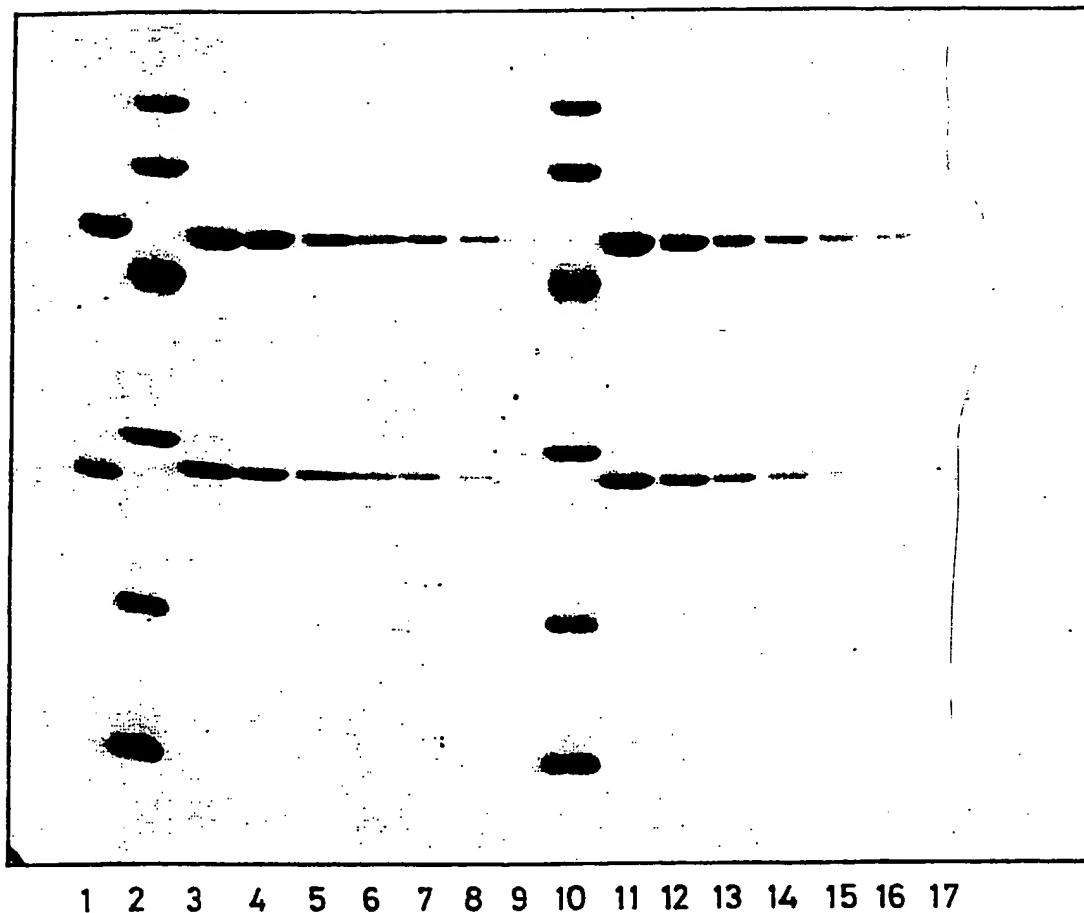


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Fig. 14 (ii)

B.



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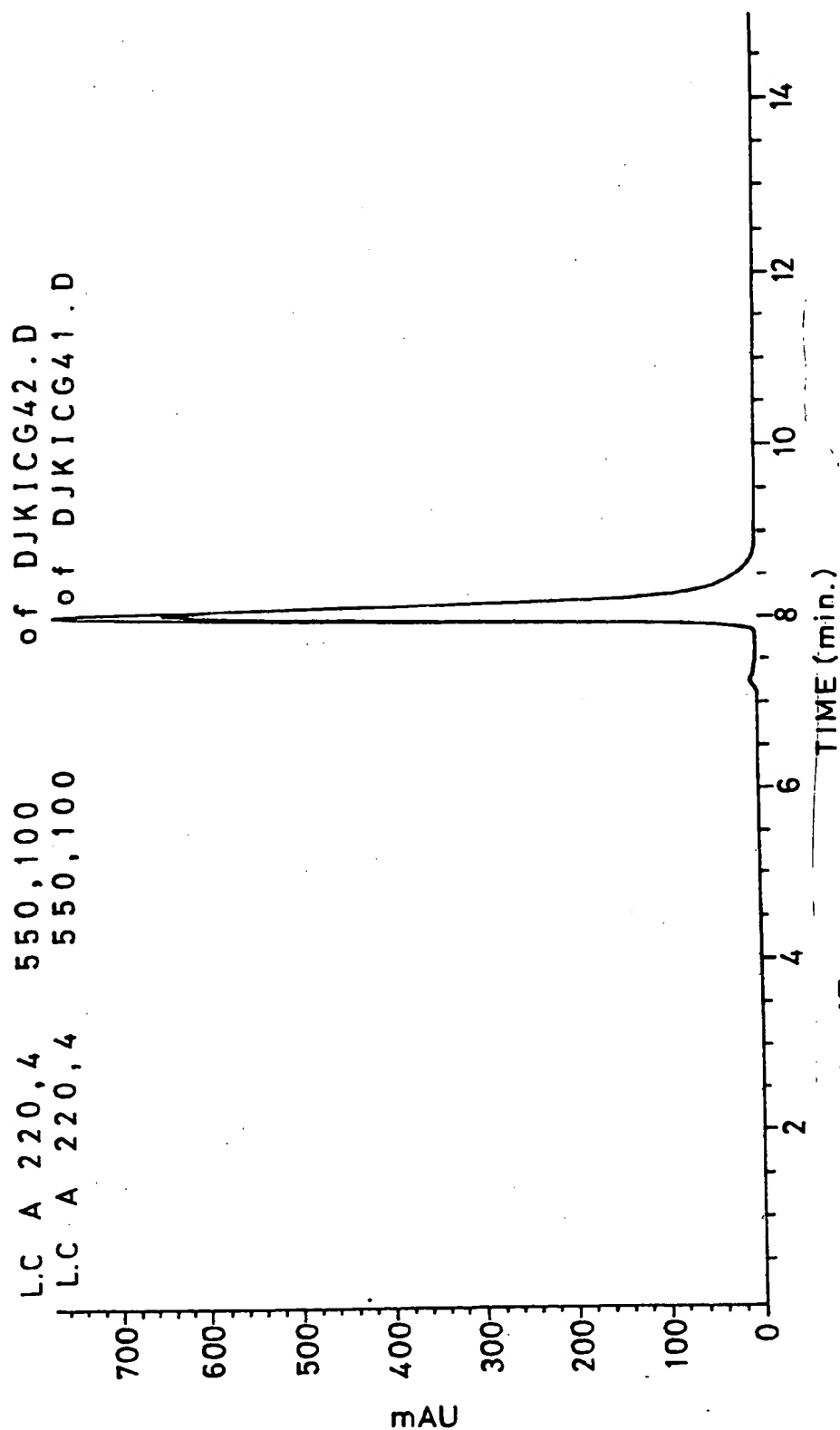


Fig. 15

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Purified anti-ICAM1 Chimeric Antibodies

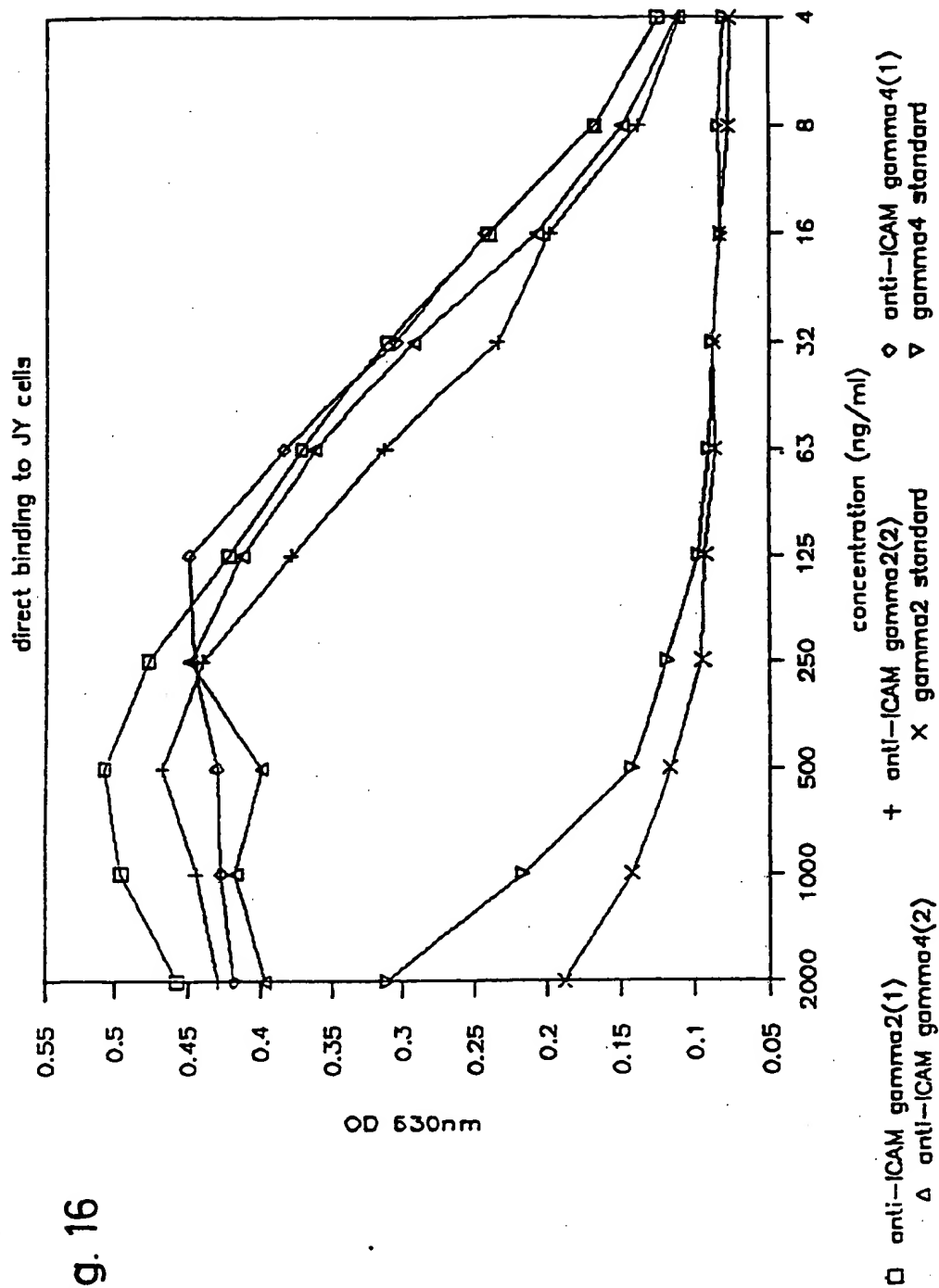


Fig. 16

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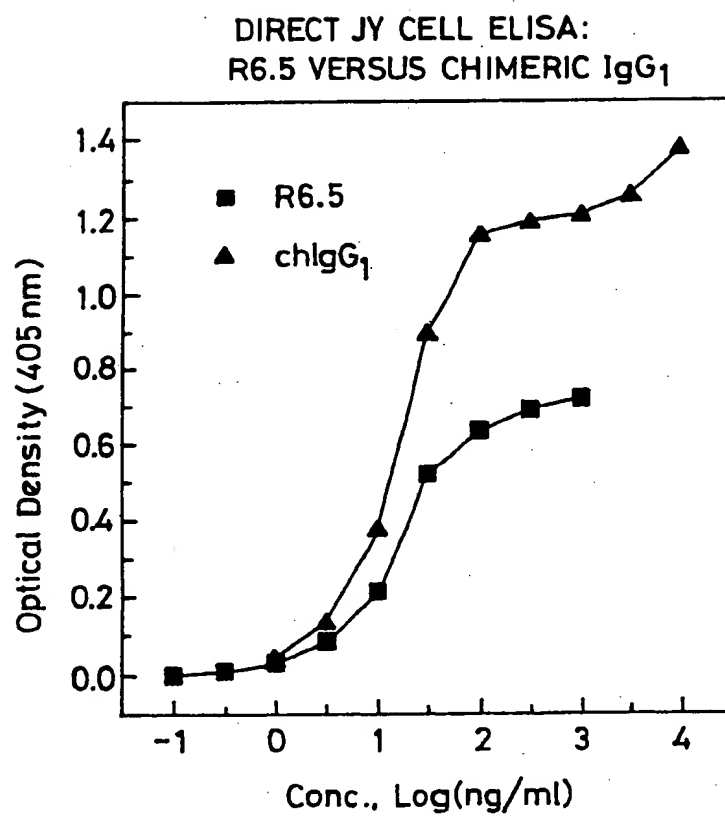


Fig. 17

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Fig. 18

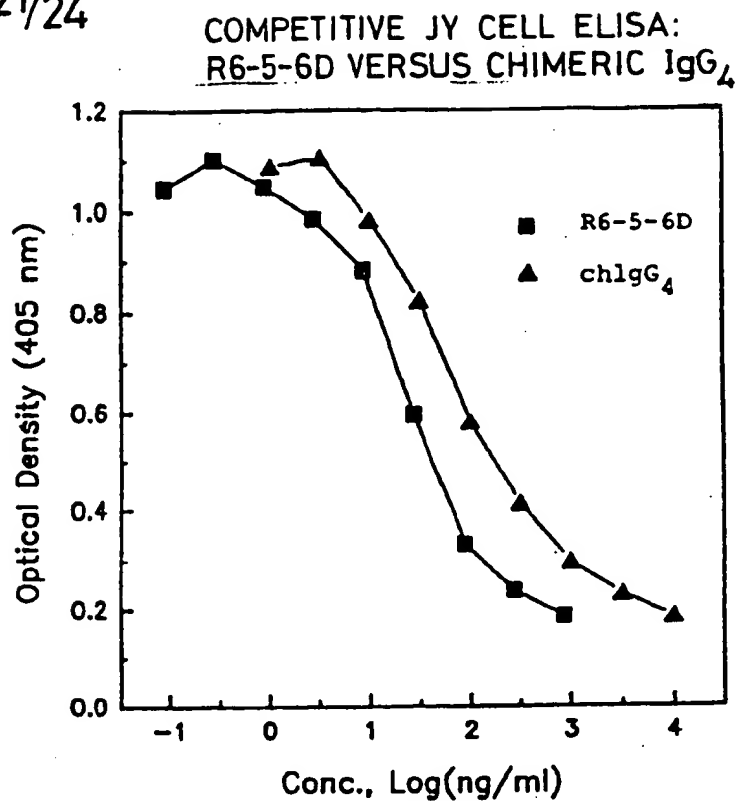
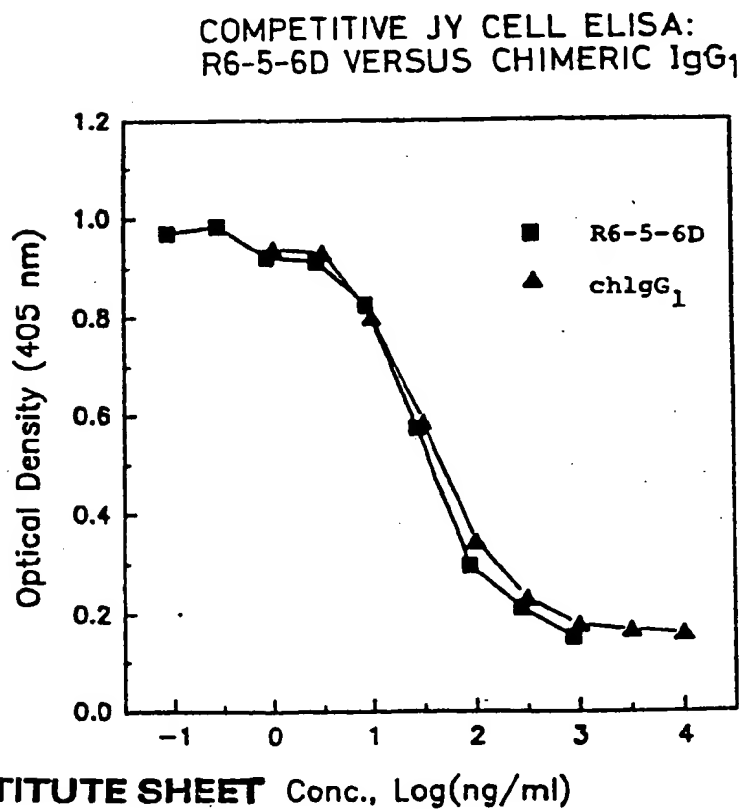


Fig. 19



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## CHIMERIC IGG2 VS. R6-5-6D, JY CELL ELISA

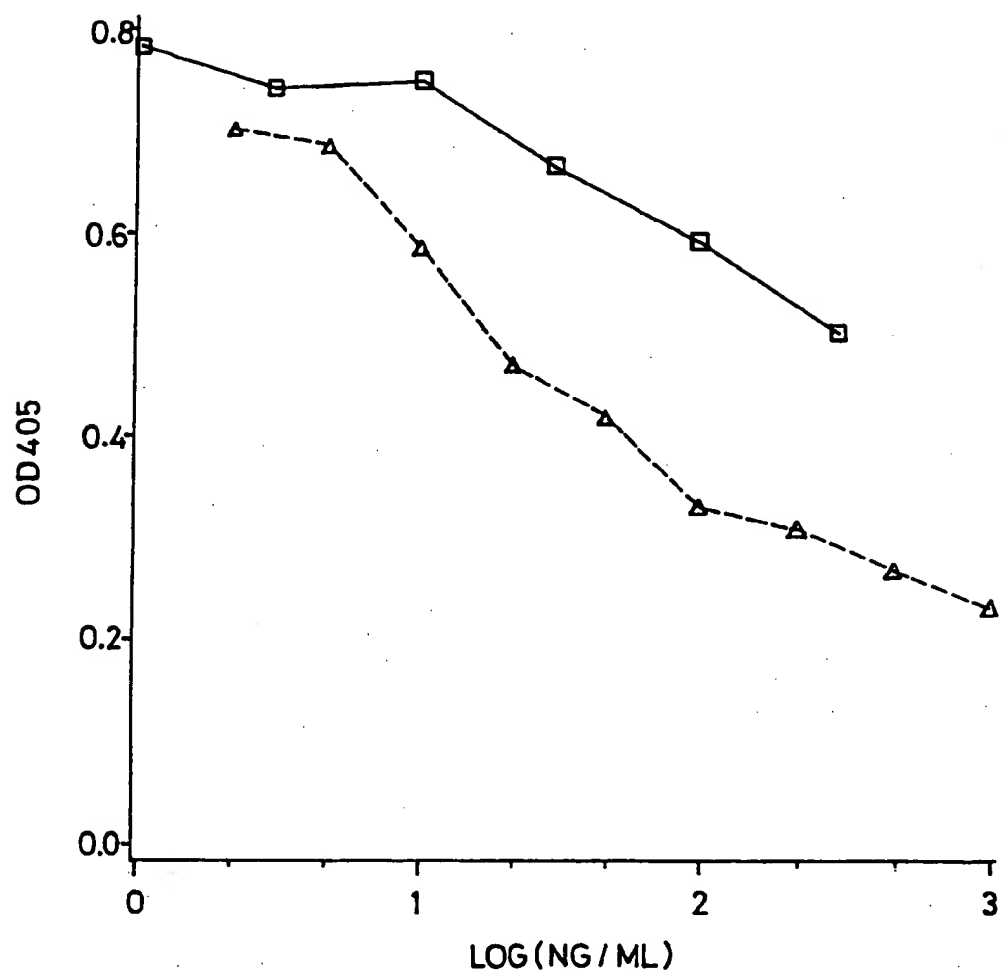
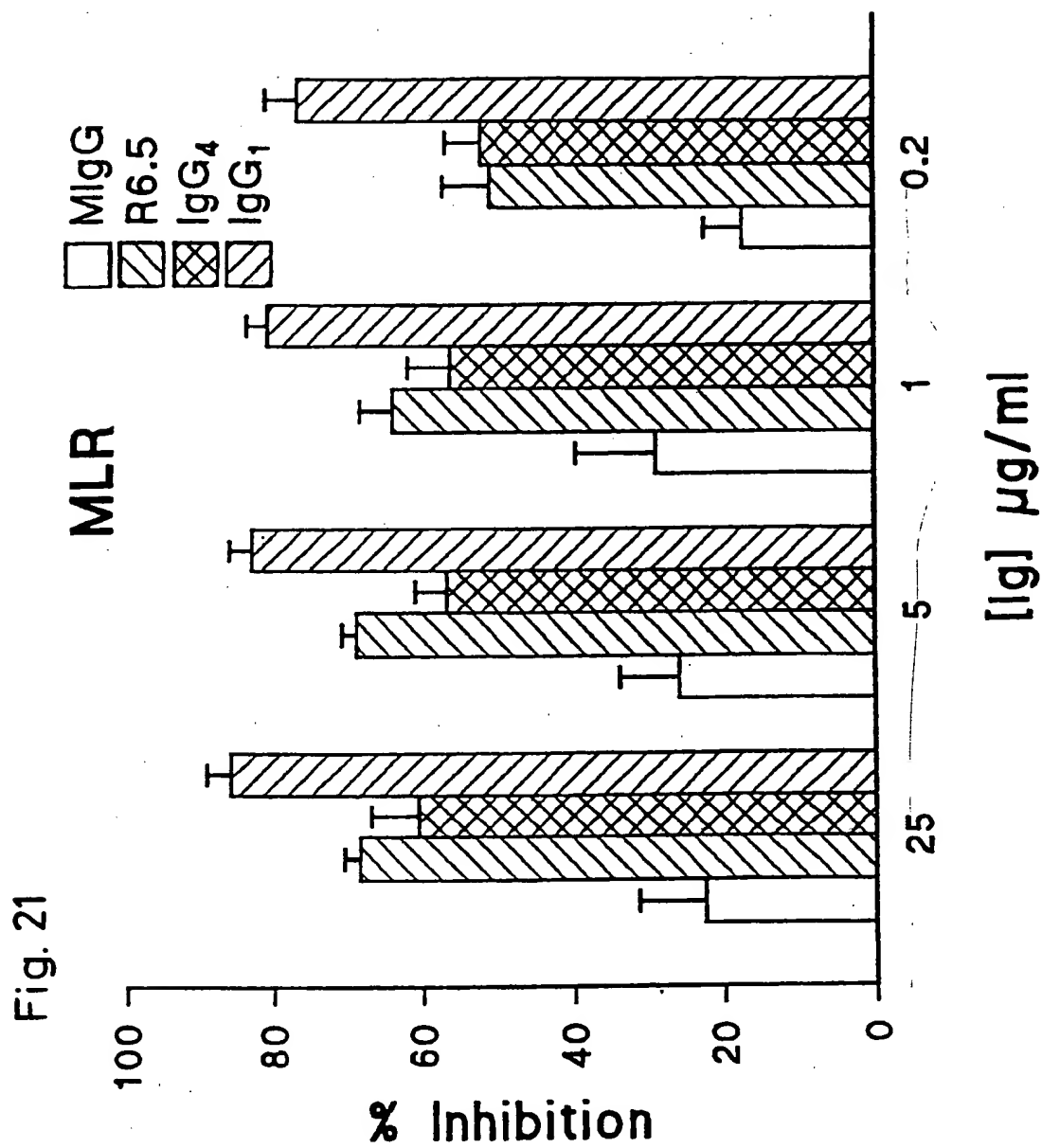
LEGEND  $\triangle\triangle\triangle$  R6-5-6D  $\square\square\square$  CHIMERIC IGG2

Fig. 20

SUBSTITUTE SHEET

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SUBSTITUTE SHEET

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INHIBITION OF VASCULAR PERMEABILITY IN A MODIFIED  
SCHWARTZMANN REACTION WITH CHIMERIC ANTI-ICAM-1 MAb

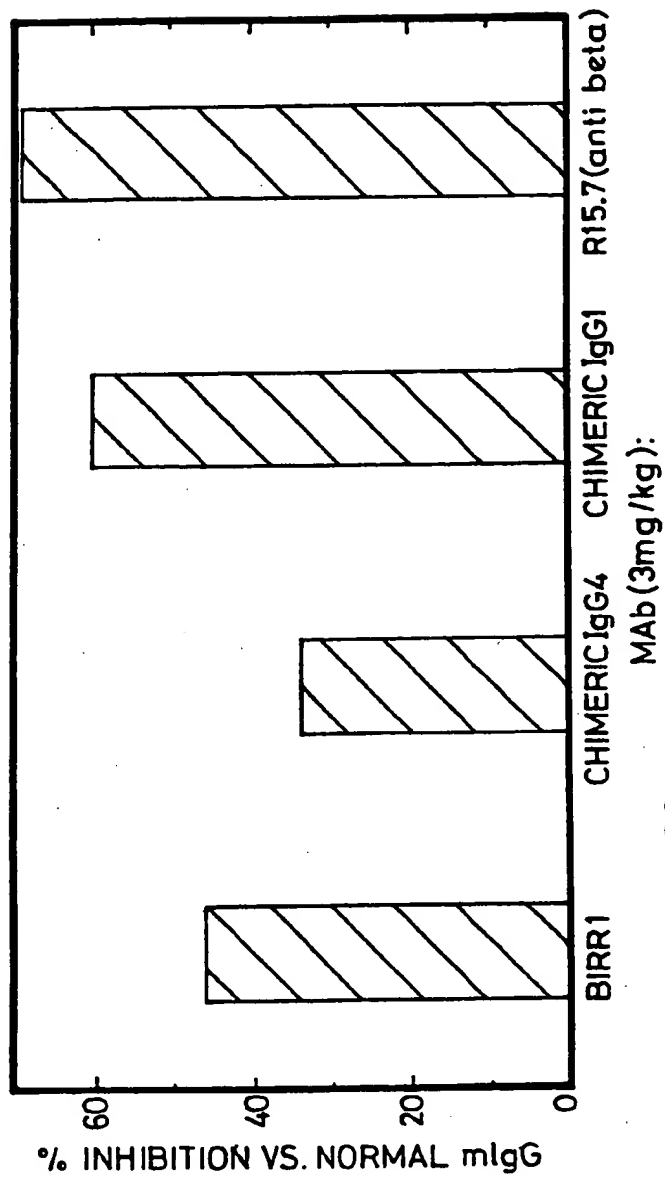


Fig. 22

# INTERNATIONAL SEARCH REPORT

International Application No. **PCT/US91/02946**

## I. CLASSIFICATION OF SUBJECT MATTER (In several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): **A61K 39/395; C07K 15/28**  
US. Cl.: **424/85.8; 530/387**

## II. FIELDS SEARCHED

Minimum Documentation Searched

Classification System	Classification Symbols
U.S.	530/387, 388, 389; 424/85.8, 88; 435.69.1, 69.3, 70.21, 172.2, 172.3, 240.27, 252.3, 252.33, 320.4

Documentation Searched other than Minimum Documentation to the extent that such documents are included in the fields searched

Databases: Dialog (File 5, 73, 155, 351, 357, 399); USPTO Automated Patent System (File USPAT, 1971-1991).

## III. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	US, A, 4,816,567 (CABILY ET AL.) 28 March 1989, See Entire Document.	1-18, 52-53
Y	JOURNAL OF CLINICAL INVESTIGATION, Vol. 82, issued November 1988, C.W. Smith et al., "Recognition of an Endothelial Determinant for CD18-dependent Human Neutrophil Adherence and Transendothelial Migration", pages 1746-1756. See Entire Document.	1-32, 48-53
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, USA, Vol. 81, issued November 1984, Morrison et al., "Chimeric Human Antibody Molecules: Mouse Antigen-Binding Domains with Human Constant Region Domains", pages 6851-6855. See Entire Document.	1-32, 48-53
Y	NATURE, Vol. 321, issued 29 May 1986, Jones et al., "Replacing the Complementarity-determining regions in a Human Antibody With Those From a Mouse", pages 522-525. See Entire Document.	1-32, 48-53

\* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claims or which is cited to establish the prior art state of another citation or other special reason (to be specified)
- "O" document relating to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the meaning of the underlying invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is considered with one or more other such documents, such combination being known to a person skilled in the art

"Z" document mentioned in the same patent family

## IV. CERTIFICATION

Date of the Actual Completion of the International Search

18 JULY 1991

International Searching Authority

IPEA/US

Date of Mailing of the International Search Report

16 AUG 1991

*Robert D. Budens*  
Robert D. Budens

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant ID/Claim No.
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE, USA, Vol. 86, issued December 1989, Quen et al., "A Humanized Antibody That Binds to the Interleukin-2 Receptor", pages 10029-10033, See Entire Document.	1-18, 52-53
Y	JOURNAL OF IMMUNOLOGY, Vol. 143, No. 4, issued 15. August 1989, Barton et al., "The Effect of Anti-Intercellular Adhesion Molecule-1 on Phorbol-Ester-Induced Rabbit Lung Inflammation, pages 1278-1282, See Abstract and Discussion.	19-32, 48-53
Y,P	EUROPEAN JOURNAL OF IMMUNOLOGY, Vol. 20, issued December 1990, Geissler et al., "A Monoclonal Antibody Directed Against the Human Intercellular Adhesion Molecule (ICAM-1) Modulates the Release of Tumor Necrosis Factor- $\alpha$ , Interferon- $\alpha$ and Interleukin-1, pages 2591-2596. See Entire Document.	19-32, 48-53
Y	WO, A, 89/01783, (CELLTECH) 09 March 1989, See Abstract.	1-17, 52-53
Y	EUROPEAN JOURNAL OF IMMUNOLOGY, Vol. 20, issued February 1990, Buckle et al., "Human Memory T cells Express Intercellular Adhesion Molecule-1 Which Can Be Increased by Interleukin-2 and Interferon- $\alpha$ , "pages 337-341, See Entire Document.	1-32, 48-53